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EFFECT OF 2,4-D ON STEM-RUST DEVELOPMENT IN OATS[1]

I. A. Ibrahim

(Accepted for publication June 1, 1951)

2,4-Dichlorophenoxyacetic acid (2,4-D) when used as an herbicide is known to cause abnormalities in some crop plants, and it may affect various host-parasite relationships or disease development as well. Although Sackston[2] reported that 2,4-D spray did not affect the relations of flax with *Melampsora lini, Colletotrichum lini,* and *Mycosphaerella linorum,* Guiscafré-Arrillaga[3] found that 2,4-D has an inhibitory action on post-harvest decay of oranges caused by *Penicillium digitatum, P. citri,* and *Phomopsis citri.* Bever and Slife[4] used concentrations of 250 to 2000 p.p.m. of 2,4-D in three different formulations to study the effect of this substance on mycelial growth of *Pythium debaryanum, Gibberella zeae,* and *Helminthosporium victoriae* on artificial media. These concentrations of the sodium and amine salts killed *P. debaryanum,* while the butyl ester form in the same concentrations only retarded growth. Growth of *G. zeae* and *H. victoriae* was retarded by these concentrations, but the organisms were not killed by any of the three forms of 2,4-D. The present writer studied the effect of 2,4-D on oat stem rust, particularly its effect on germination of urediospores and on development of rust infections on susceptible and resistant varieties of oats.

Urediospores of *Puccinia graminis avenae,* races 6 and 8, were collected by gently shaking rusted plants over a clean funnel leading to a glass vial. Germination was tested in dilutions ranging from 10 to 1000 p.p.m. of the butyl ester form of 2,4-D. A drop of the experimental 2,4-D emulsion was placed in the center of a new cover glass and surrounded by a thin ring of vaseline to keep the glass in place on a van Tieghem cell. Spores were placed in the drop by means of an inoculating needle, then the entire cell was placed in a Petri dish provided with a moist filter paper with holes of approximately the same size as the van Tieghem cell. The filter paper was moistened to maintain high humidity and to prevent evaporation from the 2,4-D emulsion. Urediospores of the two races germinated within 90 min. and their germ tubes were normal in size and shape in dilutions of 10, 20, 30, 40, and 50 p.p.m. In the concentrations exceeding 50 p.p.m. and ranging up to 500 p.p.m., urediospores of the two races did not germinate, even within 72 hr.

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station, as Paper 2284 in the Journal Series. The work was done while the author was a graduate student at the University of Minnesota and a scholar from the Government of Egypt.

[2] Sackston, W. E. The effect of 2,4-D on some diseases of flax. U. S. Dept. Agr., Pl. Dis. Rptr. 32: 9. 1948.

[3] Guiscafré-Arrillaga, J. Inhibitory action of 2,4-dichlorophenoxyacetic acid on *Penicillium digitatum* and *Phomopsis citri.* (Abstr.) Phytopath. 39: 8–9. 1949.

[4] Bever, W. M., and F. W. Slife. The effect of 2,4-D in culture medium on the growth of three different pathogenic fungi. Phytopath. 38: 1038. 1948.

Germ-tube development could be arrested by transferring the germinating urediospores from water to herbicidal concentrations of 2,4-D. Germinating urediospores of the two races with germ tubes 30 μ long were transferred from distilled water to a 500-p.p.m. 2,4-D emulsion. The germ tubes did not grow during the next 12 hr.

Immersion of rusted oat leaves in a 500-p.p.m. emulsion of 2,4-D for 24 hr. impaired only slightly the germinative capacity of the urediospores. Leaves of the Bond variety of oats rusted by races 6 and 8 were immersed in the chemical. Every 2 hr. for 24 hr. some leaves were removed and urediospores from the pustules were tested for germination. Even after the tested leaves had been immersed for 24 hr., the urediospores were not killed and 60 per cent germinated when removed from the chemical. Spores from the untreated uredia germinated 10 to 15 per cent higher than those treated with the chemical.

These facts suggest that 2,4-D used as an herbicide in fields of small grains also may be of considerable use as a protectant against rust infection. It is possible also that the herbicide might prevent or alter the development of uredia as well as prevent the germination of urediospores and growth of germ tubes.

Application of an herbicidal concentration of 2,4-D (1000 p.p.m.) to oat foliage either before or after inoculation of the oats with rust reduced the number of uredia of race 8 developing on the susceptible variety Bond. Three pots, with 15 plants in each, were prepared for each of the following nine treatments: (A) control, not sprayed; sprayed (B through E) 4, 3, 2 or 1 day before inoculation, (F) 2 days after inoculation, immediately after plants were removed from the moist chamber, and (G through I) 4, 6, or 7 days after inoculation. All plants were inoculated with race 8 at the same time. The leaves were moistened and rubbed with the fingers to remove the leaf bloom, and inoculum was brushed over the plants as evenly as possible. Inoculated plants were placed for 48 hr. in three moist chambers, each chamber containing one pot from each treatment. Twelve days after inoculation the uredia on each set of 45 seedlings were counted and counts on sprayed plants were calculated in terms of percentages of the counts on the control plants.

Plants sprayed with the 2,4-D always had fewer uredia than the control plants (Table 1). Oats that were sprayed with the herbicide 4, 3, or 2 days before they were inoculated with rust had approximately 20 per cent as many uredia as had the control plants. Whether the application of herbicide one day before rust inoculation is significantly less effective in reducing numbers of uredia is not known, but it is possible that during the moistening and rubbing of the plants in preparation for rust inoculation some of the herbicide was washed off more easily from recently sprayed plants than from those sprayed earlier. When the herbicidal spray was delayed until after rust inoculation, the 2,4-D interfered with rust develop-

TABLE 1.—*The amount of stem-rust infection produced by Puccinia graminis avenae race 8 on the susceptible oat variety Bond when 2,4-D was sprayed on the oat foliage before or after rust inoculation*

Treatment	Time of spraying with 2,4-D (1000 p.p.m.)		No. of uredia on 45 plants	Percentage of infection[a]
A	Control, not sprayed		887	100
B	4 days before inoculation		172	19
C	3 days	do	167	18
D	2 days	do	175	19
E	1 day	do	255	29
F	2 days	do	49	5
G	4 days	do	112	13
H	6 days	do	308	34
I	7 days	do	320	35

[a] Infection on the control plants was considered 100 per cent and other infections were calculated as percentages of this standard.

ment at least up to the time that rust flecks were appearing on the oat seedlings, one week after the rust inoculation. The greatest interference, of course, occurred when the 2,4-D was sprayed on the plants 2 days after they were inoculated with rust and immediately after they were removed from the moist chamber. It is possible that turgidity and water congestion of the leaves at that time enhanced the effect of the chemical, for such plants had only 5 per cent as many uredia as had the control plants.

The characteristic reactions of oat varieties to stem-rust races were not significantly altered by herbicidal concentrations of 2,4-D. Five varieties of oats representing the different types of resistance to stem rust (Andrew for the Richland type, Mindo for the White Tartar type, and Garry, Hajira × Joanette, and Victoria × (Hajira × Banner) for the Canadian type) were inoculated with races 2, 6, 7, and 8 of oat stem rust. An emulsion of 2,4-D (1000 p.p.m.) was applied at three different times: 2 days before inoculation; 3 days after inoculation; and 7 days after inoculation. Control plants, not sprayed, were used as standards for the infection types produced by the four races of rust. The development of stem rust on the five varieties was not sufficiently affected by the chemical to change a susceptible reaction to a resistant one, and vice versa, although the infection types produced by the four stem-rust races varied slightly. For example, Andrew is characteristically resistant to race 7, and the infection type 2– appeared on the control plants. On plants that were sprayed with 2,4-D, this race produced infection types 1 and 1+ which were indicative of slightly more rust resistance than was in the control plants. However, the characteristic infection type of race 8 on Garry is 1+ which was produced on the control plants, while infection types 1++ and 2– appeared on plants sprayed with 2,4-D. Thus, the chemical induced slightly more susceptibility in the sprayed plants of Garry than in the control plants.

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AUREOMYCIN CHEMOTHERAPY OF CROWN GALL IN TOMATOES[1]

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(Accepted for publication June 20, 1951)

Plant chemotherapy, the introduction of some chemical agent to the internal system of a plant to act against bacterial, fungal, or virus infections, has been explored by a number of workers. An excellent review of the field through 1948 is given by Stoddard and Dimond (14). In an attempt to use an antibiotic for this purpose, Anderson and Nienow (1) found that soybeans containing four to five units of streptomycin per ml. of expressed sap developed typical symptoms of infection when inoculated with *Xanthomonas phaseoli* var. *sojense*.

The presence of aureomycin in a plant grown with its roots in a solution of this antibiotic (3) suggested the use of this drug for phytochemotherapy. Preliminary work seemed to indicate that aureomycin-treated and nontreated Lima beans were equally susceptible to *Xanthomonas phaseoli* (Erw. Smith) Dowson (American Type Culture Collection No. 9563), the organism causing bean blight. Other work with the organism of crown gall, *Agrobacterium tumefaciens* (Smith & Townsend) Conn,[3] on tomatoes indicated a lower incidence and degree of infection in aureomycin-treated plants than in controls. Chemical treatments of plants against crown gall have been reported previously, among others, by Ark (2), Brown and Boyle (5, 6, 7), Hampton (10), and Verona (15).

The purpose of the study reported here was to see whether aureomycin could exert chemotherapeutic action on crown gall of tomatoes.

MATERIALS AND METHODS

Bonny Best tomato plants were grown in mineral culture solution in 400-ml. wide-mouth glass jars. Shive and Robbins' (13) mineral nutrient (Formula I) with added supplementary solution "A" of Hoagland and Snyder (11) was used. Each plant was held in place in a $\frac{1}{2}$-in. hole in a metal jar lid with a pad of cotton-filled gauze wrapped around the stem. To transfer a plant from one solution to another, it was necessary only to unscrew the lid and screw it in place over another jar.

The plants were grown in two different cabinets, a "light" one and a "moist" one. The former is described in a previous paper (3). Its bank of ten 40-watt, white fluorescent lamps was operated on a cycle of 14 hr. of light and 10 hr. of darkness. The moist cabinet was a box built up around

[1] Abstract of a portion of the dissertation submitted to the Graduate School of Arts and Sciences of the University of Cincinnati in partial fulfillment of the requirements for the Ph.D. degree (1951).

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[3] Culture supplied by Dr. B. M. Duggar of Lederle Laboratories.

a recess in the laboratory window utilizing a portion of the window for one wall. During the winter this tended to bring the temperature in the cabinet to 10–15° C. However, the air was warmed and held to 18–22° C. by a thermostatically controlled blower which pulled air from the room past an electrical resistance heater element and forced it into the cabinet. Humidity was raised to about 75 per cent by evaporation of water from a wet cloth hung in the cabinet, with one edge dipping into a reservoir of water. The temperature and moisture conditions for this cabinet were chosen to lie within a range conducive to tumor initiation (12).

Inoculations were made by puncturing the stem with a needle dipped in a 4-day culture of *Agrobacterium tumefaciens* which had grown at 24° C. in A.C. broth (Difco). Two or more inoculations were made in each stem, with the lowest 1 or 2 cm. from the roots and the others spaced about 1 cm. apart along the stem.

The procedure for each experiment was as follows: One-month-old plants which had been in the light cabinet were inoculated as described above and then arranged in two groups. One group, the control plants, was transferred to jars containing fresh nutrient solution. The other, the treated plants, was transferred to jars of the same solution to which Aureomycin hydrochloride (Lederle)[4] had been added. Concentrations of 5, 10, and 20 μg of aureomycin per ml. of nutrient solution were used. A preliminary experiment showed that 50 μg/per ml. was the minimum dosage which would cause severe wilting and stunting of the plants. In order to stay well below this range, the upper dosage level of these experiments was set at 20 μg per ml.

Both groups of plants were placed in the moist cabinet, to provide optimum conditions for the development of tumor cells from host cells. They were kept there 4 days, since the conversion seems to take about this length of time (4). After this period, the plants were returned to the light cabinet. When the level of the nutrient in the jars became low (about 2 weeks), both treated and control plants were transferred to plain mineral nutrient.

After 5 weeks, galls 3–20 mm. in diameter had developed at most of the inoculation points on each control plant. In general there were fewer galls on the treated plants. The diameter of each such gall was measured even where there were more than one at a given inoculation point. The galls from each group were sliced off and weighed as a group.

Three percentage ratios were computed from these data: 1) the number of inoculation sites at which galls developed on the treated plants to the number of inoculation sites which showed galls on the control plants; 2) the wet weight of galls of treated plants to that of the control plants; and 3) the sum of the cross sectional areas of all galls developed on the treated plants to that of galls on the control ones. This calculation was made assuming that the area of each gall is given by $\pi D^2/4$, where D is the

[4] Supplied by Lederle Laboratories Division, American Cyanamid Co.

measured diameter of the gall. The area ratio was chosen in preference to a diameter ratio in order to make possible a more accurate representation of gall development at inoculation points where more than one gall had developed.

RESULTS

Fewer galls developed on plants treated with aureomycin than on the controls. The galls of the treated plants were of lower average area and weight than those of the controls. The difference in appearance of the gall growth of the two groups is shown in figure 1.

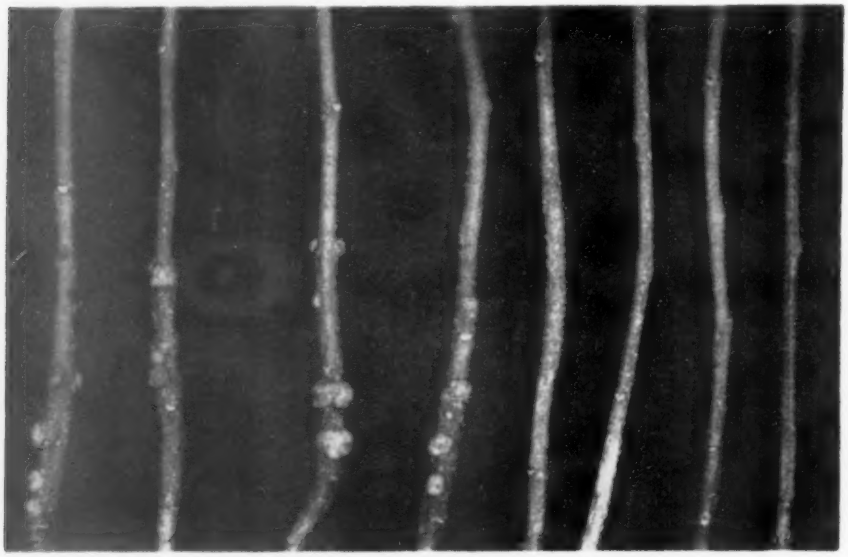

FIG. 1. Stems of tomato plants inoculated at four points with crown gall. The four on the right were grown in mineral nutrient solution containing 20 μg of aureomycin per ml. The four on the left were controls.

Confirmation of the effectiveness of the treatment is provided by the fact that all the percentage ratios described earlier are considerably less than 100 (Table 1). For the dosage of 20 μg per ml., the very small percentages, the general appearance of the galls, and the gall sizes themselves (Table 2) indicate nearly complete control. There was some browning of roots at this dosage but no noticeable wilting or difference in general appearance of the control and treated plants. Neither was there any measurable difference in heights of the control and treated plants.

DISCUSSION

In connection with previous attempts to control crown gall, it might be noted that Stoddard and Dimond (14, p. 360) point out that "there appears to be no record of crown gall being completely controlled by the introduction of materials into a plant by root absorption or by injection, the only successful treatments having been by application of chemicals directly into

TABLE 1.—*Number of inoculation sites with galls, and weight and area of galls on treated plants as percentage of those on control plants*

Concentration of aureomycin in nutrient of treated plants (μg per ml.)	No. of plants[a]	Inoculations per plant	No. of positive inoculations	Wet weight of galls	Area of galls[b]
			Per cent	*Per cent*	*Per cent*
5	14	2	77	32.0	42.0
10	6	4	37	.....	8.0
10	22	4	83	35.0	43.0
10	6	6	72	12.0	14.0
20	8	4	6	0.2	0.5

[a] In each experiment half of the plants were grown in a solution of aureomycin in mineral nutrient, the other half were grown in mineral nutrient alone.
[b] Area of cross section from $A = \pi D^2/4$ where D is the measured diameter in mm.

TABLE 2.—*Diameter of each gall developed on tomato plants grown in 20 μg per ml. aureomycin solution and on controls. Total weights of all galls in each group*

Treatment	Plant number	Diameter of galls (in mm.) at inoculation position[a] on stem:				Total wet weight of galls (in gm.)
		1st	2nd	3rd	4th	
Aureomycin, 20 μg per ml.	1	0	0	0	0	0.0036
	2	3	0	0	0	
	3	0	0	0	0	
	4	0	0	0	0	
Controls	1	7	8	10	7	1.946
	2	19	22	4	6	
	3	6	3, 8[b]	5, 7, 4, 4, 3	10, 7	
	4	3, 6	8	6	6	

[a] Numbered from root upwards. First position 1 or 2 cm. above root.
[b] Entry of two or more different diameters indicates there were that number of more or less separate swellings at the given inoculation position.

or onto the gall tissue." In the present work with aureomycin, the treatment was by root absorption and nearly complete control was obtained at a dosage of 20 μg of aureomycin per ml. of nutrient.

Throughout this work it was assumed that the principal action of the aureomycin was on the bacteria rather than on the tumor cells. Tissue culture experiments of de Ropp (8, 9) have indicated that this is true when streptomycin is used against crown gall. In view of this, inoculations and treatments were performed at the same time in the present study. This was to allow the aureomycin a chance to act on the bacteria before they had caused the completion of the conversion of host cells to tumor cells. It has been indicated that once this change takes place the tumor may continue to grow without the bacteria (16).

SUMMARY

Fewer and smaller galls developed on tomato plants grown in a solution of Aureomycin hydrochloride (Lederle) in mineral nutrient following

needle inoculation with *Agrobacterium tumefaciens* than on similar plants grown in mineral solution alone.

Concentrations of 5 and 10 μg of aureomycin per ml. of nutrient solution gave a slight reduction in incidence and extent of gall growth. A dosage of 20 μg per ml. gave practically complete control. Severe wilting, stunting, and death of the plants were caused by doses of 50 μg or more per ml.

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SEED TRANSMISSION OF RED NODE VIRUS IN PINTO BEANS[1]

W. D. Thomas, Jr., and R. W. Graham[2]

(Accepted for publication June 20, 1951)

Since the occurrence of a red-node epiphytotic in western Colorado in 1947, red node has become one of the more important diseases of pinto beans in the State (11). Preliminary observations in Colorado have indicated that seed transmission of the virus may occur, although other investigators have failed to support this view (9, 10). A severe outbreak of red node of pinto beans in the Arkansas Valley of Colorado in 1948 was suspected of being caused by infected seed from western Colorado. Reports are numerous on the seed-borne nature of viruses of legumes, including pea mosaic (1), soybean mosaic (3), Lima-bean mosaic (4), common bean mosaic (7), and others.

MATERIALS AND METHODS

Seed from varieties of pinto beans, including U. I.[3] Pintos 72, 78, 111, and 71, U. S. Pintos 1, 5, and 14, Scottsbluff Pinto, San Juan Pinto, Wyoming Pinto, and local pinto selections were used in determining the incidence of red-node virus in seed the first year after infection of the parent plants; U. I. Pinto 78 alone was used for determinations the second year after infection. The seed was obtained from plants known to have become infected with red node in experimental plots at Rocky Ford and Grand Junction, Colorado.

The seed was grown in insect-proof cages containing steam-sterilized soil having a pH of 7.3 in a greenhouse, periodically fumigated, at 24°–27° C. (2). The cages were closed for the duration of the tests, and optimum moisture relations were obtained by subirrigation with pans having rubber-tubing outlets.

Because of the difficulty in obtaining typical red-node symptoms in greenhouse plants grown from infected seed, extracts were taken from all plants expressing symptoms, and passed into virus-free pinto seedlings (U. I. 78) for positive determinations of infection. The seedlings were inoculated with fresh, crude extract just before the primary leaves were fully expanded, using a carborundum powder No. 320 as an abrasive, following the technique as outlined by Zaumeyer and Thomas (12).

SYMPTOMS IN PLANTS GROWN FROM INFECTED SEED

The first symptoms of seed-borne red node appear just before primary leaves are fully expanded. A translucent, faint rose-doree color may involve the entire pulvinus of the primary leaf, but usually is localized on

[1] Published with the approval of the Director as Paper No. 357 of the Scientific Journal Series of the Colorado Agricultural Experiment Station.

[2] Plant Pathologist and former Research Assistant, respectively.

[3] University of Idaho.

the lower side, and may easily be overlooked unless observed in diffuse light. At 27° C. this discoloration may become more intense, developing in the first node and eventually appearing in the petioles of both primary leaves. In advanced stages the discolorations extend down the stem and become oxblood red or carmine (8). The plants usually are stunted, having extremely short internodes, and finally die or break off at the infected node. At lower temperatures the original discoloration of the pulvinus usually disappears and the young leaves may have a mild chronic mottling. Such plants may mature without further symptoms of red node unless prolonged periods of high temperature and soil moisture prevail.

INCIDENCE IN PLANTS GROWN FROM CURRENTLY INFECTED SEED

To determine the incidence of red-node virus in seeds obtained from currently infected plants, 21,424 seeds were tested from 35 seed lots. Very little seed-borne infection appeared. The total average infection was 1.4 per cent and the maximum infection was 6 per cent in one sample of U. I. 72 beans. Negative results obtained with five samples were particularly interesting because other investigators have failed to obtain seed transmission of the red-node virus (11).

The evidence, supported by field observations, is that the red-node virus may be seed-borne. The amount of transmission, however, is small in plants grown from seed obtained from plants currently infected.

INCIDENCE IN PLANTS GROWN FROM SEED PRODUCED ONE YEAR AFTER INITIAL INFECTION

Pierce and Hungerford (6) found that seeds obtained from plants currently infected with common bean mosaic transmitted the virus to 33.07 per cent of the seedlings. When seed from these plants were tested, 48.6 per cent of the seedlings were infected. The results of these investigators were later confirmed by Nelson (5). Because of these findings and because of our own evidence that red node may be seed-borne in seed from plants currently infected, further experiments were made with seed from infected samples produced one year after initial infection. Such an experiment was especially important in view of the apparent recovery symptoms sometimes observed in seedlings grown from infected seed.

Seeds of Pinto U. I. No. 78 from 25 infected plants grown from infected seed were used. Testing methods were similar to those in the foregoing test; however, the severity of symptoms on parent plants was recorded as an infection index on a scale from 1 to 4, with 1 indicating no infection and 4 severe infection.

There was no apparent correlation between incidence of the disease in seedlings and the severity of symptoms in parent plants. Of 358 plants 26.5 per cent were infected. Twenty-five control plants produced by virus-free certified seed had no infection. It seems doubtful that the high incidence of infection was due primarily to insect transmission of the virus, as

the average incidence of the disease in the source plot was no greater than in the previous year. This evidence shows a striking increase in infection by the red-node virus in seed produced one year following infection of the sample. A second test with another seed sample gave similar results.

When seeds from individual plants were indexed according to their location within infected pods, no uniformity of the occurrence of the virus was observed. Seeds in the first and third positions often produced healthy seedlings, whereas infected plants arose from seeds in the second and fourth positions. Similar observations on common bean mosaic have been reported (5).

RELATION BETWEEN SEED COLOR AND SEED TRANSMISSION OF VIRUS

Since the red-node virus may be transmitted through seed, attention was given to the color patterns of seed from currently infected pods with the hope that these might prove to be a means of recognizing those carrying infection.

Twelve classes were established as a result of preliminary observations of color markings. The seeds were first classified under white and then under ultraviolet light.[4] From 10 varieties and lines 3,105 seed beans were separated into the 12 color classes and planted in the greenhouse. Infection was determined by transferring extracts of the resulting seedlings to test plants grown from certified Pinto U. I. No. 78 seeds.

Only 47 seedlings of 3,105 (1.5 per cent) in this test were confirmed as carrying the red-node virus. Four samples, which contributed 1,380 seeds to the test, produced no infected seedlings. One sample had 11.6 per cent infection; other samples had less. Due to an insufficient population of infected seedlings these data do not offer any conclusive evidence on the correlation of color with infection.

DISCUSSION

The faint red-node symptom characteristic of incipient infection by the seed-borne virus could easily be overlooked either in the field or in the greenhouse. In direct sunlight the discoloration is difficult to see. Numerous field observations have shown that, unless the proper conditions of high temperatures and moist soil exist, the incipient symptoms of red node may disappear and not show again until later in the season. They may be replaced by recovery symptoms characterized by mottling of the younger leaves. Because of this, it may be possible for red node to increase in the field and escape observation until the following season.

The increased percentage of transmission characteristic in seed produced one year after initial infection emphasizes the importance of maintaining a low tolerance level in a seed certification program. The Colorado Seed Certification Program has accordingly placed a 5 per cent tolerance on pinto beans registered for certification.

[4] A 250-watt GE-mazda mercury arc lamp, type A–H5, was used with a Switzer Bros. filter No. 41 transmitting light with wave length between 3253Å and 4200Å.

SUMMARY

Red-node symptoms resulting from seed transmission of the virus were expressed as a translucent, faint rose-doree discoloration at the base of the pulvini. In the absence of optimum conditions for symptom expression, these symptoms may be replaced by recovery symptoms expressed by a mottle of young leaves.

Limited seed transmission (average, 1.4 per cent) of the red-node virus was observed in 21,424 plants grown from seed produced by plants currently infected with red-node virus. Seed from infected plants grown from infected seed produced an increased amount (average, 26.5 per cent) of the disease.

There was no uniformity in the occurrence of the virus within the seeds in individual pods or among different pods on infected plants. The presence of the virus within infected seeds could not be detected by seed color or markings.

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CELLULAR AUTONOMY IN CROWN GALL

ARMIN C. BRAUN

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The author concluded (1) as a result of studies on tumor inception and development that the continued rapid and abnormal proliferation of crown-gall tumor cells in periwinkle (*Vinca rosea* L.) becomes completely independent of the inciting bacteria 4 days after inoculation. This finding has been fully confirmed by Klein[1] and others (2, 3). Riker and collaborators (2) urged caution about too broad an application of this view because, they state, "In the secondary galls of sunflower, the crown gall bacteria frequently die without heat treatment. Such galls commonly have stopped developing in the plant."

Some of the most rapidly growing autonomous[2] types of tumor cells with which the author has worked have been isolated from bacteria-free petiolar secondary tumors of the sunflower (*Helianthus annuus* L.). Other instances have been noted, however, in which the petiolar tumors have developed very slowly and remained small in the host. The sunflower is an annual and the plants mature in 80 days. Isolation of tissue from the interior of the petiolar tumors is often difficult. These tumors are not large, and pieces of tissue isolated from them for cultural studies are of necessity very small. Because of their small size, mechanical injury to the cells during isolation may often be considerable. Many of the petiolar secondary tumors of sunflower do not therefore represent suitable material with which to work.

A condition that appeared comparable to that observed in the sunflower has been produced experimentally in the periwinkle (1). This host is a perennial and can be maintained in an actively growing condition for prolonged periods. It therefore appeared to be a more suitable plant than the sunflower for studying the question of autonomy in small, slowly developing tumors.

The methods used in this study are the same as those described previously (1). Large, woody periwinkle plants were inoculated at several hundred points with the Brown peach strain of the crown-gall bacterium. The inoculated plants were permitted to remain on a greenhouse bench at about 25° C. for a desired period of time, after which the host-parasite complex was subjected to a thermal treatment of 46–47° C. for 4 days.

[1] Dr. Richard M. Klein (personal communication) carried out heat treatment experiments on periwinkle and confirmed the fact that not only are all living crown-gall bacteria destroyed by thermal treatment, but that "Contamination of the isolation media was not excessive, none to occasional bacterial or fungal colonies developing per plate. The contaminants appeared to be primarily air-borne and the fungal types were those found normally in the laboratory." Dr. Klein used potato dextrose agar, Patel's agar and peptone dextrose broth for isolation purposes.

[2] The term autonomy as used here refers not only to the ability of bacteria-free cells to grow more or less profusely and indefinitely on a culture medium free of added growth substances, but also to the capacity of those cells to grow and multiply in an uncontrolled manner in a host plant.

Following heat treatment, the plants were held on a greenhouse bench for periods ranging up to 8 months.

Numerous isolations made from representative material at various periods after inoculation demonstrated that all viable crown-gall bacteria had been eliminated from the host by the third day of the thermal treatment. Isolations made with the use of beef extract peptone dextrose agar during the late stages of the heat treatments were commonly free of fungal

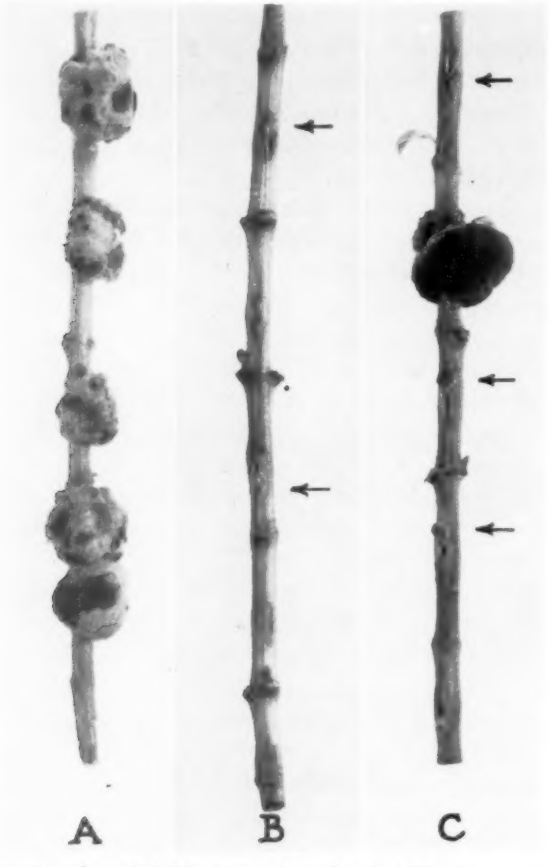

FIG. 1. Segments of periwinkle stems inoculated with the crown-gall bacterium. The bacteria were permitted to act on the host at about 25° C. for: A, 4 days; B, 34 hr., before being destroyed by the thermal treatment. C shows the two types of response on the same stem segment. The pictures were taken about 10 weeks after heat treatment. Mag. × 1.0. (Photographs by J. A. Carlile.)

contamination.[1] The thermal destruction rate of crown-gall bacteria in the host (1) is in accord with the findings obtained in studies dealing with the *in vitro* killing of pure cultures of the crown-gall organism at a temperature of 46.5° C. (2). In view of these facts, it seems entirely unnecessary to postulate (2) the presence of fungal staling products in the tumors in order to account for the destruction of the bacteria as reported in these and other studies (1, 2).

The results of the experiments to be discussed here are summarized in

the accompanying figures. These studies demonstrate that when the crown-gall bacteria were permitted to act on the host cells for 4 days at 25° C. before being eliminated by the thermal treatment, the resulting overgrowths developed rapidly into large neoplasms (Fig. 1, A). When, on the other hand, the bacteria were destroyed by heat 34 hr. after inoculation, very small overgrowths resulted at some points of inoculation (Fig. 1, B). The stem segments shown in figure 1, A, B, C, were photographed about 10 weeks

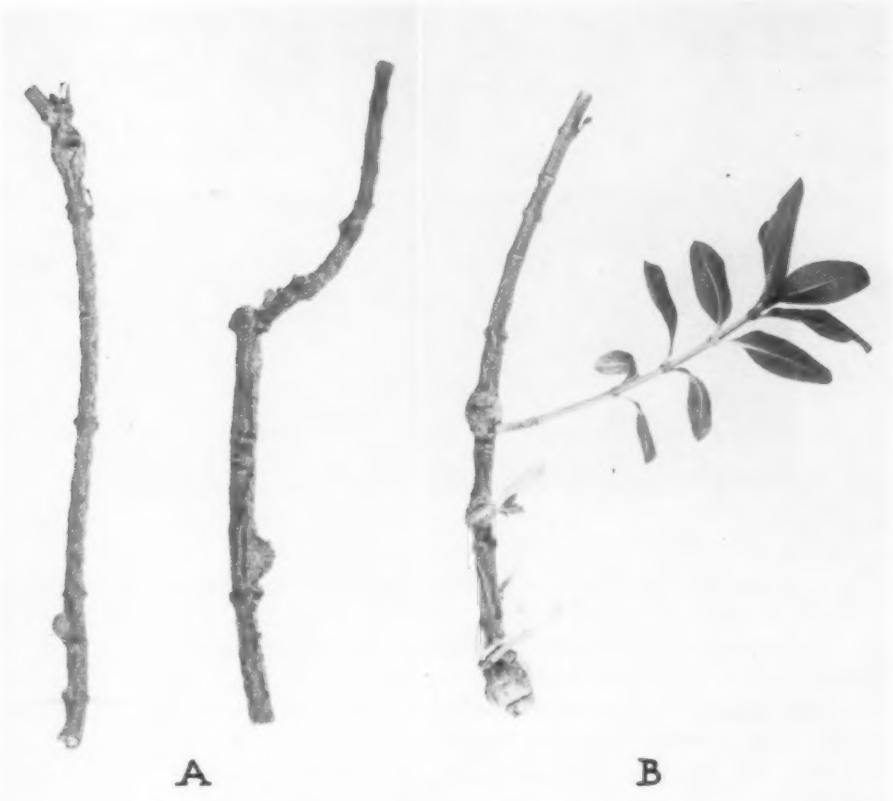

FIG. 2. Segments of periwinkle stems in which the bacteria were permitted to act for 34 hr. before being destroyed. The pictures were taken 8 months after the heat treatment. Some of the incipient galls continued to develop very slowly during the 8-month period. Their growth was stimulated somewhat by the presence of an axillary bud in the vicinity of the overgrowth, as shown in B. Mag. × 0.38. (Photographs by J. A. Carlile.)

after the bacteria had been destroyed by heat. At that stage incipient galls like those shown in figure 1, B appeared to have stopped developing in the host. Their growth habits seemed to be similar to those of certain of the petiolar secondary tumors described above. Yet some of the small overgrowths in periwinkles continued to develop very slowly and after 8 months reached a moderate size (Fig. 2, A). When an axillary or adventitious bud developed in the vicinity of such tumors, their growth rate was increased, due presumably to the diffusion of a stimulating material from the actively

growing shoot (Fig. 2, B). That these differences in developmental potentialities are real and not a function of variation in the host has been shown by testing the two types of response on the same stem (Fig. 1, C).

Fragments of tissue isolated from five of the slow-growing tumors developed more slowly on White's culture medium than did comparable fragments of tissue isolated from cells transformed by the bacteria in a 4-day period. Two clones of each type of tissue were maintained in culture for almost 5 years. They retained their characteristic growth habits throughout that period. The change from a slow-growing to a fast-growing type of tumor cell may occur spontaneously in culture, however, and instances have been observed in one of the clones in which fast-growing cells developed from the slow-growing stock. The relative growth rates of these cell types are shown in figure 3. Fragments of the slow-growing tumor, when im-

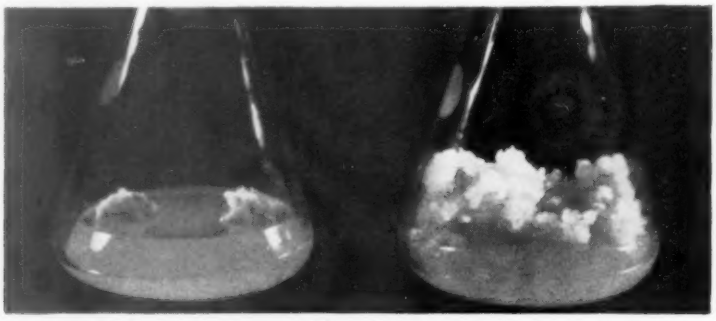

FIG. 3. The relative rate of growth in culture of tumors initiated by the bacteria in 34 hr. and 4 days is shown. (Photograph by J. A. Carlile.)

planted into a healthy host, developed after a period of time into overgrowths of moderate size.

These results demonstrate that both slow- and fast-growing tumors of the periwinkle are capable of autonomous development. To account for the observed differences in the growth rate of tumor cells, it is suggested that a high rate of growth in tumor cells is a property that is acquired gradually in the 4-day period during which the alteration of normal to tumor cell is fully accomplished.

If, as the evidence suggests, cellular transformation in crown gall takes place gradually, then it appears likely that a degree of cellular change is reached during the earliest stages of transformation when the affected cells are not sufficiently altered to permit their proliferation to become an automatic process. At that early stage the affected cells may not be autonomous.

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YELLOW DOT, A VIRUS DISEASE OF BEAN

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Snap beans grown in experimental plots at Hermiston, Oregon, and Prosser, Washington, are often severely mottled and stunted by viruses other than curly-top virus (*Ruga verrucosans* Carsner & Bennett). Certain varieties and hybrids frequently have a reddish-brown pod blemish, either separately or in combination with mottle and stunting. Red-node virus (*Annulus orae* H. var. *phaseoli* Thomas & Zaum.) and yellow bean mosaic virus have been isolated from such plants. In August 1949 at Prosser, Washington, a virus obtained from a bean plant with a systemic mottle and pod blemish produced in the greenhouse veinal necrosis on rubbed leaves of Pinto bean U.I. No. 78, as well as very mild systemic mottle and subsequent yellow leaf spotting. The same virus was isolated from alfalfa near Twin Falls, Idaho, in August 1950. The original infected bean leaves contained what was tentatively identified as yellow bean mosaic virus as well as the new virus. The new virus was separated out by passage through tobacco; the yellow bean mosaic virus, by passage through tepary bean (*Phaseolus acutifolius* A. Gray). The causal agent of the pod blemish occurring on the bean plant from which these two viruses were isolated has not been determined.

The new virus has been previously described as a strain of alfalfa mosaic virus (11). This paper presents studies on host range, properties, and cross protection. The disease is referred to as yellow dot and the virus is given the trinomial *Marmor medicaginis* H. var. *phaseoli* var. nov.

MATERIALS AND METHODS

All investigations were made in the greenhouse at Beltsville, Maryland, at temperatures from 21° to 27° C. The virus was maintained on bean, Pinto U.I. No. 78, and the Samsun variety of Turkish tobacco. Virus-infected bean leaves from plants inoculated with expressed juice from the leaves of the diseased plant from Prosser, Washington, were desiccated and stored at 8° C. The virus maintained on the plants was occasionally compared with the virus isolated from the desiccated virus-infected leaf tissue to determine whether any contamination had occurred or if any mutants had appeared.

Carborundum powder No. 320 was used as an abrasive in all transfers. All plants were inoculated in the early stages of growth. The symptoms on most of the plants in the host range studies were described from plants grown 3–6 weeks in 4-in. pots. When symptoms were not visible on plants in the host range studies, an attempt was made to recover the virus by inoculating Pinto U.I. No. 78 or tepary beans.

The viruses used in the cross protection tests were obtained as follows: Tobacco ring-spot (*Annulus tabaci* var. *virginiensis* H.) and tobacco-streak (*Annulus orae* H.) from H. H. McKinney, and Ladino clover yellow-patch (*Marmor medicaginis* H. var. *Ladino* Kreitl. & Price) from K. W. Kreitlow, Plant Industry Station, Beltsville, Maryland; alfalfa virus X from F. P. McWhorter, Oregon State College; and strain C of cucumber mosaic virus from J. P. Fulton, University of Arkansas. The red-node virus was originally obtained from naturally infected Pinto bean plants collected near Greeley, Colorado.

SYMPTOMS AND HOST RANGE

Bean. Veinal necrosis appeared within 4 days after inoculation on leaves of Pinto, Blue Lake, McCaslan Pole, and Kentucky Wonder varieties (Fig. 1, A) and was most pronounced on the under surface. Occasionally a few necrotic primary lesions, 1–3 mm. in diameter, developed on the inoculated leaves. Lesions were sometimes solid but more frequently a small green center was surrounded by several small, concentric, dark-brown necrotic rings somewhat similar to those produced by tobacco ring-spot virus. A reddish discoloration of the petiole, nodes, and stem, considerably milder than that produced by the red-node virus, occurred next. Plants were stunted but continued to grow. Young leaves of old infected plants had a very faint mottle and a few small bright-yellow spots, 1–2 mm. in diameter (Fig. 1, B). Varieties such as Tendergreen and Topcrop developed extremely mild mottle and a few small yellow spots, but no necrosis. The mature plants were only slightly stunted and produced a normal number of pods. Infected Red Kidney and Bountiful plants were not mottled, but after several weeks had a few small yellow leaf spots. No pod symptoms have been observed on any variety inoculated.

Tobacco. Symptoms were seldom produced on inoculated leaves of the Samsun variety of Turkish tobacco. Infected plants were slightly stunted and sometimes a few faint yellow spots, 1–3 mm. in diameter, developed on older leaves after 7–10 days.

Nicotiana glutinosa L. and *N. rustica* L. had symptoms similar to those of Turkish tobacco, but more yellow spotting occurred on the latter.

Datura stramonium L. Infection was difficult to obtain. No primary symptoms developed on the inoculated leaves and a few chlorotic spots on the older leaves were the only systemic symptoms observed.

Gomphrena globosa L. Numerous small reddish spots with whitish centers developed on inoculated leaves (Fig. 1, E). These were frequently followed by systemic yellow stippling of new growth.

Cucumber, var. Cubit. Yellow circular spots, 1–2 mm. in diameter, usually developed on the inoculated cotyledonary leaves. Later, large, irregular, yellowish blotches appeared on the trifoliate leaves and caused distortion and puckering (Fig. 1, D).

Tepary bean. Numerous solid necrotic lesions, 2–3 mm. in diameter,

developed within 3 days on inoculated leaves (Fig. 1, C). No systemic infection occurred.

Cowpea, var. Blackeye. Numerous small brick-red primary lesions developed; some systemic necrotic spotting, mottle, leaf distortion, and severe stunting followed.

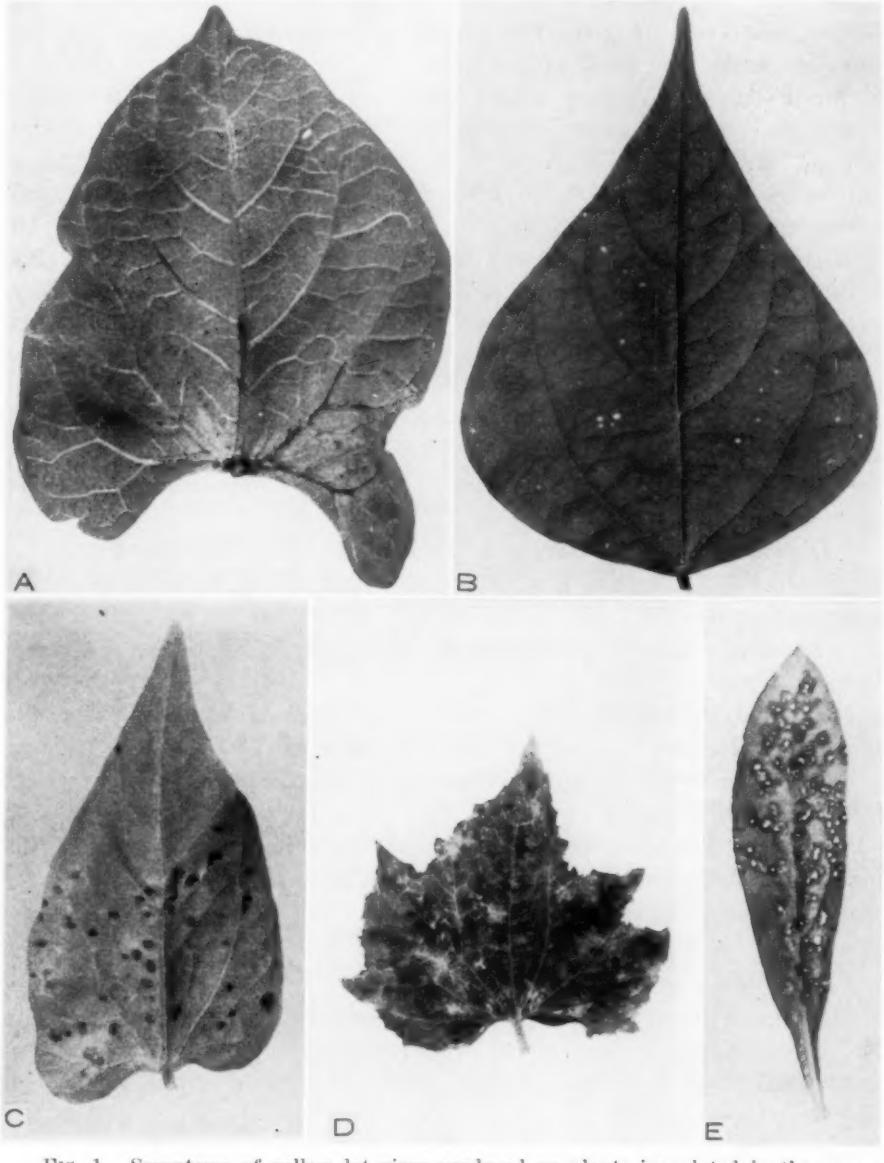

FIG. 1. Symptoms of yellow-dot virus produced on plants inoculated in the greenhouse. A. Veinal necrosis on rubbed primary leaf of Pinto U.I. No. 78 bean. B. Yellow spotting on trifoliate leaf of Early Blue Lake bean. C. Local lesions on rubbed primary leaf of tepary bean. D. Yellowish blotches and puckering on leaf of Cubit cucumber. E. Primary lesions on rubbed leaf of *Gomphrena globosa* L.

Pea, var. Little Marvel. Extremely mild leaf mottle.

Zinnia, var. Sunshine Tint. Symptomless, or slight veinclearing and mild leaf mottle.

Other bean varieties: Idaho Pinto, Pinto U.I. No. 78, Great Northern U.I. No. 59, 81, and 123, Red Mexican U.I. No. 3, Early Blue Lake, Blue Lake 231, Kentucky Wonder Wax, Burger's White Kentucky Wonder, Robust, and Tenderlong No. 15—primary veinal necrosis; petiole and stem necrosis; later a few small yellow spots.

Brittle Wax, Contender, Flight, Giant Stringless Green Pod, Improved Commodore, Keeney's Stringless Green Refugee, Improved Kidney Wax, Logan, Longreen, Slendergreen, State, Sure Crop, Tendergreen, Topcrop, Unrivalled Wax, and U. S. No. 5 Refugee—mild mottle followed by small yellow spots.

Black Valentine, Bountiful, Cherokee, Light Red Kidney, Dark Red Kidney, Dwarf Horticulture, Florida Belle, Full Measure, Idagreen, Keystonian, Landreth Stringless Green Pod, Michelite, Pencil Pod Black Wax, Plentiful, Pure Gold, Sensation Refugee No. 1066, and Supergreen—a few small yellow spots.

Other Leguminosae: Phaseolus aconitifolius Jacq., *P. aureus* Roxb., *P. angularis* Wight, *P. calcaratus* Roxb., and *P. mungo* L.—veinal necrosis of the inoculated leaf, sometimes followed by very few inconspicuous yellow spots on upper leaves.

Melilotus alba Desr., *Phaseolus coccineus* L., *Trifolium incarnatum* L., *T. pratense* L., *T. repens* L. f. *giganteum* Lagr.-Foss, *Medicago sativa* L., and *Glycine max* (L.) Merrill—mild leaf mottle.

Phaseolus lunatus L. var. Triumph—difficult to infect; only a few yellow spots on leaves.

Crotalaria spectabilis Roth, *Cyamopsis tetragonoloba* (L.) Taub., *Lupinus albus* L., and *Sesbania* sp.—solid necrotic local lesions only.

Vicia faba L. var. Broad Windsor—numerous necrotic primary lesions followed by mild mottle.

Pepper, var. California Wonder; potato, U.S.D.A. seedling 41956; tomato, var. Marglobe; beet, var. Detroit Dark Red; and cabbage, var. Jersey Wakefield—no infection.

PROPERTIES

Expressed juice from young systemically infected leaves of Pinto U.I. No. 78 was used for the physical property studies. The tepary bean was used in all property studies and the results were based on the presence or absence of local lesions.

The virus was inactivated between 62° and 64° C. in 10 min. It withstood dilution of 2000×, but not 3000×, and was infectious after storage for 72 hr. *in vitro* at 18° C., but not after 96 hr. The virus was recovered from desiccated bean leaves after storage for 1 year at 8° C.

SEED TRANSMISSION

Seeds from yellow-dot-infected bean plants of several varieties growing in the greenhouse were harvested and planted to determine whether the virus was seed-borne. No infection was obtained on 539 plants obtained from the seeds.

CROSS PROTECTION

Topcrop bean plants systemically infected with yellow-dot virus were inoculated with the following viruses: tobacco ring-spot, tobacco streak, red-node, Ladino clover yellow-patch, alfalfa-N, and strain C of cucumber mosaic virus which produces necrotic symptoms on beans.

Primary lesions characteristic of tobacco ring-spot, tobacco streak, and red-node viruses developed on the inoculated leaves previously infected with the yellow-dot virus. Eventually systemic necrotic symptoms of these viruses developed on these plants as well as on those inoculated with the cucumber mosaic virus. No local lesions were produced on the leaves inoculated with alfalfa-N and Ladino clover yellow-patch viruses.

Tobacco plants systemically infected with yellow-dot virus were inoculated with the Ladino clover yellow-patch and alfalfa-N viruses. No systemic symptoms, typical of the last two viruses, developed on these plants and it was impossible to reisolate these two viruses. The check plants which were inoculated with yellow-patch and alfalfa-N viruses developed symptoms typical of each virus. These results suggest that yellow-dot virus is related to Ladino clover yellow-patch and alfalfa-N viruses, but is not related to the other viruses investigated.

DISCUSSION

Studies of host range, properties, and cross protection show that the yellow-dot virus is in the alfalfa virus group. The virus is distinctive in its inability to produce on bean the local necrotic lesions characteristic of other strains of alfalfa virus as reported by Berkeley (1), Kreitlow and Price (6), McWhorter (7), Oswald (8), Johnson (3), Pierce (10), Black and Price (2), and Zaumeyer (12). The very mild systemic symptoms produced by this virus on tobacco are also distinctive.

The yellow-dot virus may be distinguished from other viruses previously described. The white clover virus described by Zaumeyer and Wade (14) produces symptoms on bean not unlike those of yellow-dot virus. The properties of the two viruses are similar. They differ, however, in that the following hosts are insusceptible to white clover virus, but susceptible to the yellow-dot virus: *Phaseolus acutifolius, P. angularis, P. calcaratus, Soja max* (L.) Piper (*Glycine max* L. Merrill), and *Vigna sinensis* (Torner) Savi. The pea mottle virus (*Marmor efficiens* Johns.) isolated from white clover by Johnson (4) produced light yellow spots and clearing of veins on bean. This virus, however, has properties different from those of

yellow-dot virus and was not infectious to *Nicotiana glutinosa* and *N. tabacum*. The white clover virus reported by Johnson (3) as a strain of alfalfa mosaic virus produced necrotic spots on the inoculated leaves and a subsequent systemic mottle in Stringless Green Refugee bean. This virus was not mechanically transmitted to zinnia or cucumber. The white clover virus described by Pierce (10) is similar to yellow-dot virus in its reported resistance to aging, the thermal inactivation point, and symptoms produced on bean. However, no infection on soybean or tobacco by the white clover virus was reported.

The severe mosaic of pea described by Johnson and Jones (5) was infectious to alfalfa and caused mild mottling of bean somewhat similar to that caused by yellow-dot virus. It differed from yellow-dot virus in not infecting tobacco and in having different properties. Yellow bean mosaic virus isolates studied at Beltsville, Maryland, frequently produce small bright-yellow spots in addition to the more extreme yellow leaf mottling considered typical.

Yellow-stipple virus (*Marmor flavopunctum* Zaum. & Thomas) (13) produces symptoms on bean very similar to those produced by the yellow-dot virus. This was particularly true on the dry bean varieties on which veinal and stem necrosis was followed by mild mottle and small, yellow, chlorotic spots. The yellow stippling caused by yellow-stipple virus was primarily confined to the dry bean varieties, whereas yellow-dot virus produced thinly scattered yellow spots on all varieties tested. The yellow-stipple virus was not infectious to any host outside of the Leguminosae. Its properties were quite different; it was infectious at 5000 × dilution and after heating to 72° C.

The symptoms of yellow-dot virus on Red Kidney resemble the heritable chlorophyll deficiency in bean, "yellow spot," described by Parker (9). This occurred in an advanced generation selection from a cross of Stringless Green Refugee × Wells' Red Kidney, as well as in a strain of Wells' Red Kidney.

Yellow dot was selected as a common name for the disease discussed in this paper instead of some term relating to the extremely mild symptoms, because yellow dot was a symptom common to all bean varieties and to most other hosts, regardless of whether primary symptoms occurred or systemic development was mild or severe.

Because of the extremely mild symptoms of the yellow-dot virus on many bean varieties it might be overlooked in combinations with other viruses. The recovery of the virus from naturally infected beans in Washington and from alfalfa in Idaho suggests that the virus may be general throughout the Western bean-growing area. It is unlikely, however, that the virus will be a serious economic factor on bean because of its mild effect on this host and its nontransmission by seeds.

TECHNICAL DESCRIPTION

Marmor medicaginis Holmes var. **phaseoli,** var. nov.

Common name: Yellow-dot virus. Hosts: Leguminosae—*Phaseolus vulgaris* L., bean; *Medicago sativa* L., alfalfa. Transmissible experimentally also to many species of dicotyledonous plants, including: Amaranthaceae—*Gomphrena globosa* L., globe amaranth; Cucurbitaceae—*Cucumis sativus* L., cucumber; Leguminosae—*Glycine max* (L.) Merrill, soybean; *Trifolium repens* L. f. *giganteum* Lagr.-Foss., Ladino clover; *Pisum sativum* L., pea; Solanaceae—*Nicotiana tabacum* L., tobacco.

Geographical distribution: Idaho and Washington.

Induced disease: No necrotic local lesions produced on bean as typical of type strain. In some bean varieties, veinal necrosis of inoculated leaves followed by mild mottle of new growth; subsequent development of thinly scattered small bright-yellow dots. In other varieties no primary symptoms, only an extremely mild mottle and yellow dots. In tobacco and *Nicotiana glutinosa* L., no symptoms, or slight stunt with a few yellowed areas.

Transmission: By inoculation of expressed juice in dilution up to 2000 ×, but not at 3000 ×.

Thermal inactivation: Between 62° and 64° C. in 10 min. Aged at 18° between 72 and 96 hr.

Immunological relationships: Infection with the type protects bean from production of local lesions by superinfection with Ladino clover yellow-patch and alfalfa-N viruses, but not from primary and systemic infection by tobacco ring-spot, tobacco streak, and red-node viruses or systemic infection by a necrotic strain of cucumber mosaic virus.

SUMMARY

A virus causing primary veinal necrosis followed by petiole and stem discoloration on most dry bean varieties, accompanied by an extremely mild mottle in some varieties and subsequent development of thinly scattered small bright-yellow dots on all bean varieties has been isolated from a naturally infected bean plant in Washington and from naturally infected alfalfa in Idaho.

Plants in the Amaranthaceae, Cucurbitaceae, Leguminosae, and Solanaceae were suscepts. There was no evidence of seed transmission in bean. No resistance was found among 45 bean varieties artificially inoculated.

The virus was inactivated between 62° and 64° C. in 10 min. and at 18° in 72–96 hr. It withstood dilution of 2000 ×, but not of 3000 ×.

Inoculation of bean with yellow-dot virus protects against infection by Ladino clover yellow-patch and alfalfa-N viruses but not against tobacco ring-spot, tobacco streak, and red-node viruses or a strain of cucumber mosaic virus causing necrosis of bean.

From similarity of properties and cross protection tests it is concluded that the yellow-dot virus is a strain of alfalfa mosaic virus. Because of the

absence of necrotic local lesions which are produced by other alfalfa virus strains on bean and the systemic mottle produced by the yellow-dot virus on this host, a variety distinction is warranted. The trinomial *Marmor medicaginis* H. var. *phaseoli* var. nov. is proposed.

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DELAYED SPORULATION OF COLLETOTRICHUM ON SOYBEAN[1]

LOIS H. TIFFANY

(Accepted for publication June 29, 1951)

Anthracnose, one of the minor diseases of soybeans, is commonly found in all major soybean-producing areas in the United States. Although it is not responsible for severe losses in yield in plantings of the present standard varieties, the use of agronomic and genetic techniques to obtain crop selections that are better adapted for specific uses and growing seasons in the various sections of the country has made anthracnose of potential importance. Since earlier investigations of the disease are in disagreement as to the time the host plants were susceptible to attack, a more intensive examination of the development of the parasite in relation to the host was undertaken.

Soybean anthracnose caused by a species of Colletotrichum was first reported from China (5) in 1919 and the organism involved was named *Colletotrichum glycines* Hori. The following year, Wolf and Lehman (11) found the same fungus in North Carolina on soybean. Later they (7) reported an ascogenous fungus, a Glomerella that they considered to be the perfect stage of *C. glycines* Hori on overwintered diseased soybean stems. They regarded it as a distinct entity, not a member of the *Glomerella cingulata* (St.) Sp. & von S. complex, and described it as *G. glycines*. The same workers described soybean anthracnose as a disease that might attack the plant at any stage of growth, but that was most destructive at the time the plant was approaching maturity. They observed fruition only on stems and pods, the affected portion being thickly covered with irregularly placed acervuli. Other workers found infection limited to the same parts. The fungus has been reported as causing the premature death of plants in the field and inhibiting seed formation after early infections.

Recently, Ling (8) found the organism to be an important factor in seedling killing, either before or soon after emergence.

Morphological and pathological studies (4, 6) have indicated that *Colletotrichum glycines* is indistinguishable from *C. truncatum* (Schw.) Andrus & Moore, the causal agent of Lima-bean anthracnose. The soybean pathogen is therefore included in this species. This organism, *C. truncatum*, is not the conidial stage of *Glomerella glycines*. In addition to *Colletotrichum truncatum*, two other *Colletotrichum* species are commonly found on soybean in Iowa, and Bain (1) has reported a leaf spot of this host associated with a straight-spored Colletotrichum, not *C. glycines*.

MATERIALS AND METHODS

The original isolates of *Colletotrichum truncatum* that were used throughout the pathological studies were obtained from Lincoln soybean in

[1] Journal Paper No. J-1863 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1179.

September 1945. All studies, both greenhouse and field, were made on the variety Lincoln.

Conidia for inoculations were obtained from 5- to 7-day-old cultures on sterilized soybean stems. Suspensions of the conidia in sterile distilled water were sprayed on the plants with an atomizer. Field inoculations were made about 8 in the evening. Following greenhouse inoculations, plants were kept in the moist chamber for 36 hr., then removed to the greenhouse bench. In seed inoculation experiments, seeds were immersed in a spore suspension, the suspension poured off immediately, and the seeds then planted in nontreated greenhouse soil.

Observations of germination of conidia and penetration by hyphae were made either on strips of epidermis from cotyledons that had been sprayed with a spore suspension and placed in a moist chamber, or on mounts of whole leaves that had been similarly inoculated, and cleared at various intervals in a saturated water solution of chloral hydrate. The details of penetration and subsequent establishment of the young mycelium were observed most clearly with a stain of cotton blue in lactophenol.

Freehand sections were used to determine the development of the mycelium within the plants. The sections were cleared 5–6 hr. in a solution of equal parts of 95 per cent ethyl alcohol and glacial acetic acid. They were stained with acid fuchsin in lactophenol, then washed and mounted in clear lactophenol. In some cases cotton blue was also used to stain the young hyphae.

Portions of the same material that was sectioned were surface-sterilized and placed on potato-dextrose agar to establish definitely that only *C. truncatum* was present.

OBSERVATIONS

In 1948, inoculations were made on plants in the field on June 27 when only the unifoliate leaves were well expanded. Later inoculations were made on additional plants on July 28. Isolations of *C. truncatum* were made on August 11 and August 27 from symptomless, apparently normal trifoliate leaves of plants inoculated June 27; also, on August 27, from material inoculated July 28. The fungus was isolated from 70 per cent of leaves collected at random from inoculated plants. At the time of the last isolations, pods were well developed.

Freehand sections made from the field collections showed that the mycelium was confined to the epidermal cells, usually lying close to the inner cell wall.

As the plants approached maturity and leaves began to fall during the second week in September, irregularly scattered areas of acervuli appeared over the entire central stem and on some of the pods (Fig. 1, A). Most of the fruiting structures on the pods were in an area where the ovule had failed to develop, but *C. truncatum* was readily isolated from the tissues of

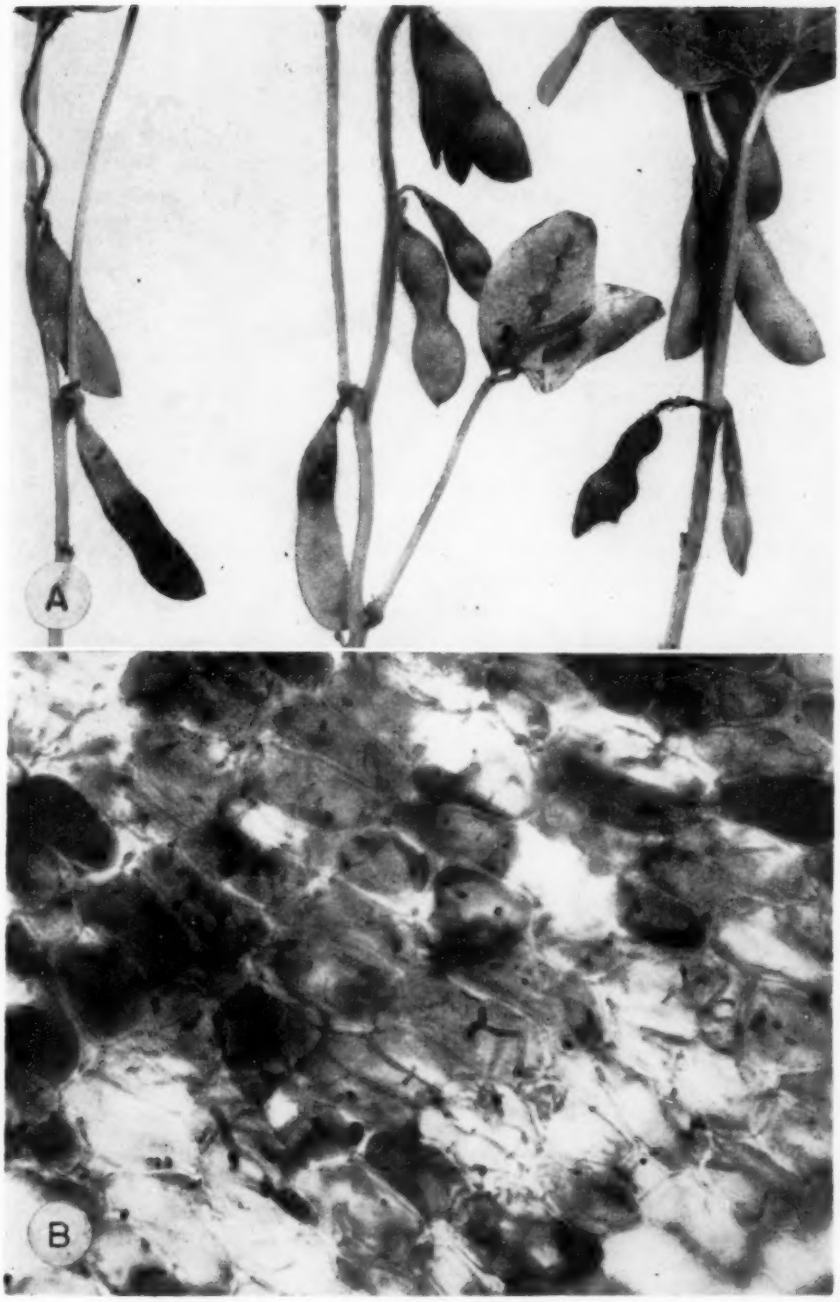

FIG. 1. A. Anthracnose on stems and pods of soybeans grown in the field. B. Mycelium of *Colletotrichum truncatum* in a soybean cotyledon (95×).

fully developed adjacent seeds in the same pod, as well as from apparently normal seeds from pods that showed no visible infection.

More critical studies were carried out in the greenhouse where the plants could be handled more easily and environmental conditions were partially controlled. Two main lines of inquiry were undertaken: 1) the mechanism of penetration and the subsequent development of mycelium in above-ground parts of the plants, and 2) the development of the fungus from infected seed.

Conidia of *C. truncatum* germinated typically by the formation of a short hypha from one end of the spore. Frequently the conidium was divided by a central cross wall at the time of germination. Conidia were also commonly found that had formed germ tubes at each end of the spore, either with or without the laying down of a cross wall. More rarely, spores formed a centrally located germination hypha as well as the two terminal ones.

Large, dark-colored appressoria which adhered tightly to the plant part were formed when the hypha touched a solid surface (Fig. 2, A). From the lower surface of the appressorium, possibly through a definite pore, an infection peg was formed that penetrated directly through the cuticle and cell wall. Entrance through stomata was never observed. The infection peg was fine, and as can be seen in figure 2, B, the mycelium widened out gradually within the host cell.

After initial penetration, further development of the mycelium depended upon the plant part entered, the age of that part, and environmental conditions. When inoculated plants were held in a greenhouse moist chamber, the mycelium developed rapidly throughout the leaf tissue both inter- and intra-cellularly. By 4 to 5 days after inoculation, the mycelium within or in a mass just above the epidermal cells had begun to form parallel groups of conidiophores interspersed with young setae. These structures were visible as well organized acervuli by the fifth or sixth day, and constituted the first external evidence of disease. Their emergence was followed by rapid disintegration of the leaf cells.

Under field conditions mycelium in the leaves produced no external evidence of infection. It penetrated the epidermal cells of leaves of any age and became established there intracellularly, but did not grow farther into the mesophyll cells. Its presence incited no visible reaction in the invaded cells, but its further development could be stimulated by placing such plants in the moist chamber.

In either greenhouse or field, the mycelium appeared to grow rapidly throughout an entire cotyledon once penetration was accomplished. The establishment of the mycelium resulted in a rapid breakdown of individual cells after a short time (Fig. 1, B). The invaded cotyledon soon had a watersoaked appearance, and subsequently dried and shriveled rapidly. Further development of cotyledonary infections will be discussed later.

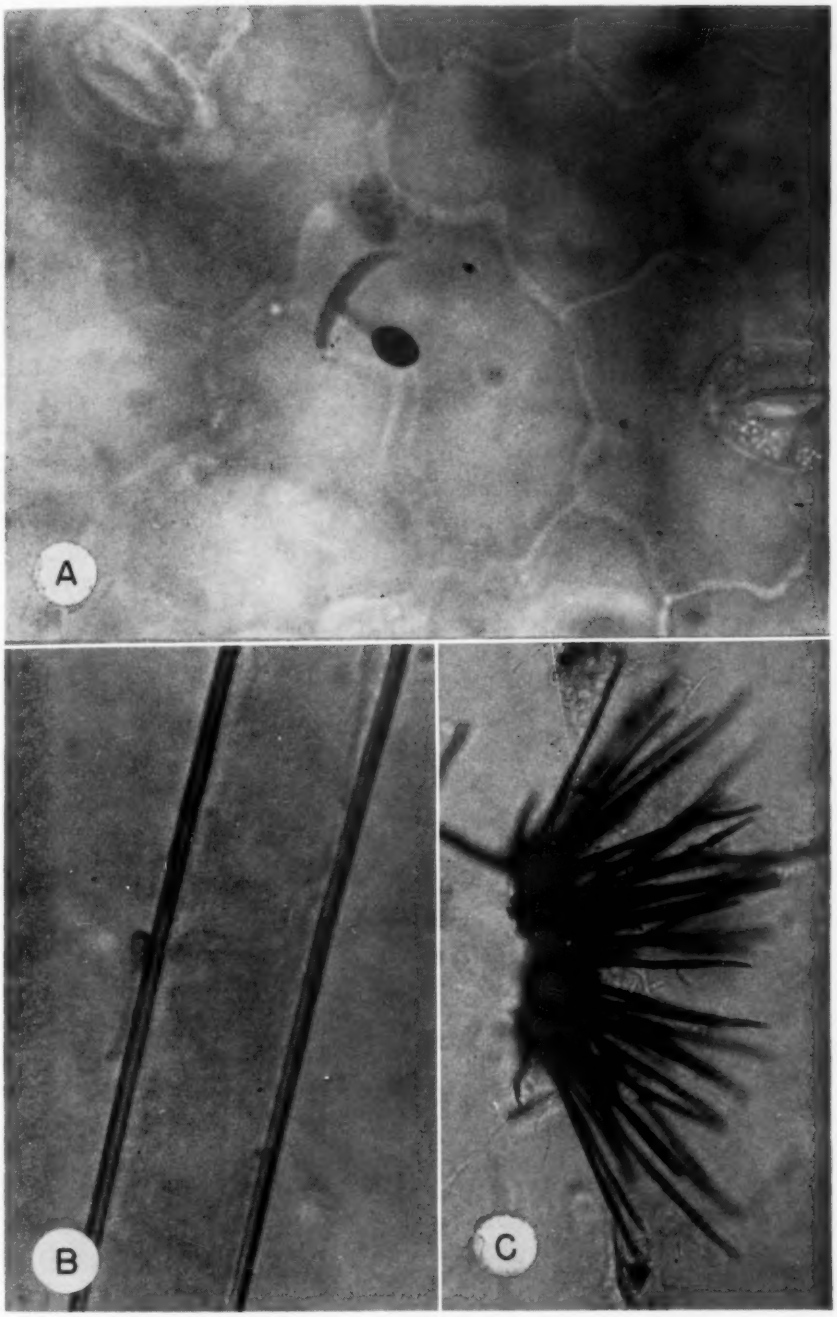

FIG. 2. A. Germinating conidium of *Colletotrichum truncatum* (362×). B. Penetration of a soybean leaf hair (362×). C. Acervulus of *C. truncatum* (95×).

Seed inoculation with conidia of *C. truncatum* resulted in three types of infection in both field and greenhouse: 1) pre-emergence killing, 2) seedling blight, and 3) symptomless establishment of internal mycelium.

In pre-emergence killing, mycelium penetrated the young embryo as soon as it resumed growth and spread rapidly. It was present in all parts of the embryo and cotyledons in 3 or 4 days, after which the tissues broke down in watery rot and were rapidly invaded by saprophytic soil organisms.

When initial invasion occurred in the cotyledons, the plants sometimes emerged and developed normally for a short time. In typical seedling blight, the mycelium ramified throughout the cells of the cotyledons and in the cortical cells of the stem which were disintegrated in a soft watery rot. From there it had spread rapidly, both radially and longitudinally, and soon girdled the young plant. After the mycelium was established in the vascular tissues and the pith, the plant collapsed. It was never found more than $\frac{1}{2}$ in. below the cotyledonary node at this stage of growth, but was established in the young leaves and the growing point after the initial girdling of the stem. The mycelium had grown much more slowly in diseased plants that had emerged from the soil, so that individual plants that developed disease symptoms were not always killed immediately. The brown spindle-shaped stem lesion was obvious from the time of emergence; however, many of the seedlings had well developed unifoliate leaves before the stem was completely girdled.

In those plants in which invasion had occurred a short time prior to emergence, or even in those in which breakdown of the cortical cells was in an advanced stage, the invaded cells had dried out rapidly resulting in characteristic sunken lesions at or just below the cotyledons. In two plants the lesion was visible for approximately two-thirds of the circumference of the stem at the time the unifoliate leaves were developing. The plants were 6 in. high with a pair of well expanded trifoliate leaves before the stem was completely girdled and the plant collapsed.

The third type of infection, that of establishment of latent internal mycelium without the development of disease symptoms, was accomplished by mycelium from the cotyledons. The cotyledons soon dried to shriveled masses of mycelium and broken cell walls that adhered to the stem for varying periods of time. The cells in the outer three or four rows of the cortex adjoining the cotyledon were killed soon after the entry of the mycelium. The collapsed walls of these dead cells formed a corky area on the stem after the cotyledons had sloughed off.

For some undetermined reason, there was no visible effect of the presence of the mycelium upon the cortical cells that were subsequently invaded. The disrupted outer cells that had been invaded directly from the cotyledons adjoined other cortical cells that were apparently normal but in which the mycelium was established intracellularly (Fig. 3, A, B). Both

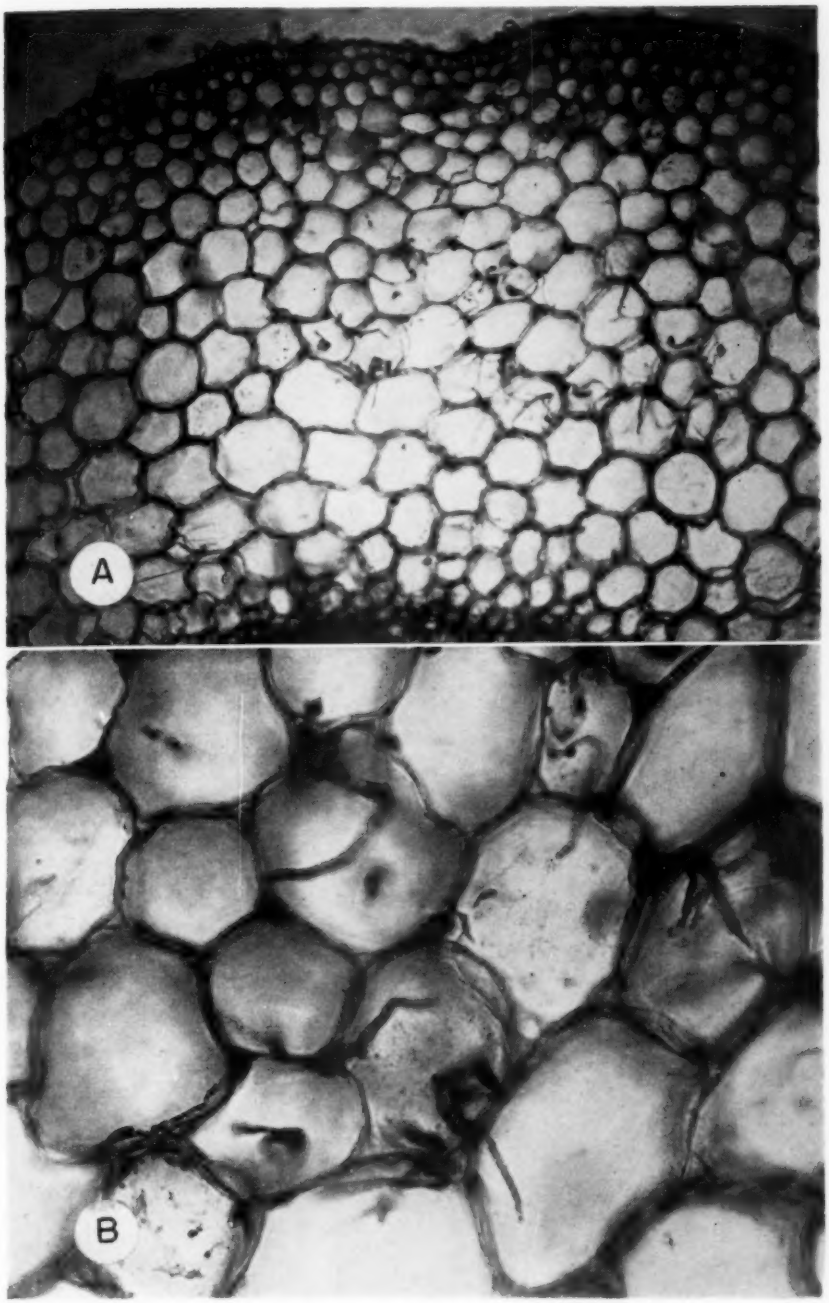

FIG. 3. A. Cross section of an apparently healthy soybean stem with mycelium of *Colletotrichum truncatum* in cells of the cortex (95×). B. Group of cortical cells from A (150×).

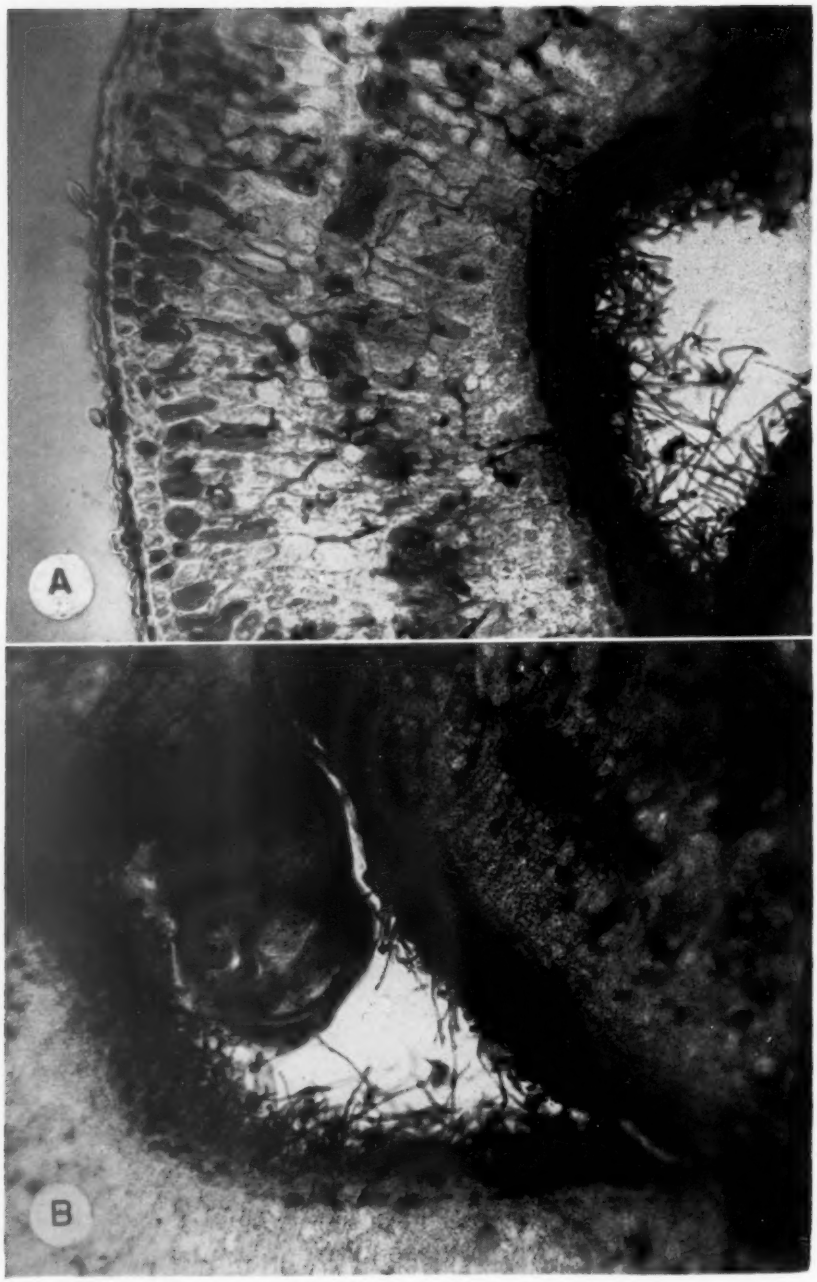

FIG. 4. Longitudinal sections of young soybean pods (42×). A. Mycelium in cells of the carpel wall. B. Mycelium in the ovarial locule.

cytoplasm and nuclei were normally distributed in invaded cells. The mycelium was fairly uniform in diameter and sparsely branched. No marked constrictions were found in the hyphae near the points of penetration of cell walls, either between epidermal and cortical cells or between adjacent cortical cells. The diameters of the original infection hyphae and the internal hyphae were approximately the same. Several hyphae were frequently found in a cortical cell, but the cells were never packed with hyphae at this stage of development as were the cells of the cotyledons.

The mycelium advanced most rapidly longitudinally. In one plant where infection had occurred from only one cotyledon, the hyphae had extended 2 mm. distally in the stem from the original infection area in $1\frac{1}{2}$ months, but was not established in the cells on the opposite side of the stem. The mycelium grew very slowly during the period of rapid development of the host plant, remaining localized in the area of original entry from the cotyledons until a week or ten days prior to flowering. At this time the mycelium grew distally in the cortical region. In several plants the progress of the hyphae could be followed from the stem through the petioles and into the blades of the lower leaves. Here the hyphae seemed to be limited to the epidermal cells, just as they had been when the original infection occurred in the leaves.

The mycelium had advanced 2 or 3 cm. below the original point of entry at this time, but did not progress farther. No mycelium was found in the roots.

When pods started to develop, mycelium was found in the cells of the carpel wall (Fig. 4, A) and in the enlarging ovarial locule (Fig. 4, B). Hyphae were also present in the cotyledons of the developing seeds. These pods had no external evidence of disease at this time. As they approached maturity, however, some became covered with acervuli while others, although infected, gave no indication of the presence of mycelium.

DISCUSSION

In several of the fruit anthracnoses (9, 10) the mycelium established between the cuticle and the epidermal cell wall remained dormant until the fruit approached maturity. However, latent mycelium in the vegetative portions of the host plant has not been considered an important factor in the development of anthracnose. Edgerton and Carvajal (3) discussed the establishment of the mycelium of *C. falcatum* between the cuticle and epidermal cells of sugar-cane leaf sheaths and leaves without external evidence of its presence, but did not mention its possible importance in the development of disease.

When apparently healthy stems of soybean plants that had grown from diseased seed were placed in a moist chamber, acervuli developed rapidly over the entire surface. Similar development of acervuli occurred when leaves from spray-inoculated plants were held in a moist chamber for 24 hr. In both cases the fruiting structures had formed from mycelium that had

been located in the epidermal cells of the leaves or in the cortical cells of the stems. In fruit latent infections (9, 10) only the cuticle was actually involved in the original invasion, for the mycelium remained localized outside the epidermal cells during the dormant stage; however, in the soybean plants the mycelium was actually present inside the cortical cells. Cox (2) has found mycelium in the xylem of Lima-bean plants that had been hypodermically injected with a spore suspension of *C. truncatum*, but in these studies no evidence was found of its presence in soybean vascular tissue.

Knowledge of the capability of the mycelium of *C. truncatum* to remain dormant in host cells and to grow extensively in some cells of the plant without disrupting their normal activities helps to explain several puzzling details of the development of anthracnose on soybean. Often no visible evidence of the presence of the disease would occur throughout the entire growing season, but as the plants approached maturity the stems were completely covered with scattered acervuli. The simultaneous appearance of these fruiting structures over the entire stem is quite expected if one considers their appearance merely a result of the resumption of active development by mycelium already present in the cells. Similarly, the presence of acervuli on lower branches that were broken early in the growing season can be interpreted as the further development of mycelium that was already established.

One of the puzzling developments in the greenhouse, the appearance of acervuli on pods that had formed on plants grown from diseased seed, can be explained by the presence of mycelium within the plant. There had been no opportunity for pod infection from external sources, therefore the growth of mycelium within the plant itself is an expected explanation. The mycelium of *C. truncatum* was capable of rapid destructive growth in both very young and mature host cells, but its success as a pathogen is linked with its ability to penetrate into other cells without appreciable disruption of their normal activities.

An important phase of this ability pertains to the role of latent mycelium in the survival of *C. truncatum* from one season to another. With the successful establishment of the mycelium inside the seed, possibly in the cells of the cotyledons, the fungus is no longer dependent for survival upon infection each succeeding year by spores produced on overwintered diseased plant materials.

SUMMARY

Seed-borne inoculum of *C. truncatum* was responsible for three types of infection on soybean: 1) pre-emergence killing, 2) seedling blight, and 3) symptomless establishment of internal mycelium. The third type was investigated in detail. Mycelium from infected cotyledons became established in the cortical cells of the stem without apparent effect on them and remained localized in the immediate stem area until flowering time. It then

resumed growth and penetrated the lower stem, petioles, leaves, and the developing seeds and pods, without the immediate development of disease symptoms. At the host's maturity under proper environmental conditions, the fungus may fruit abundantly on stems and pods.

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INHERITANCE OF RESISTANCE TO VERTICILLIUM WILT IN A TOMATO CROSS[1]

LESTER SCHAIBLE, ORSON S. CANNON,[2] AND VICTOR WADDOUPS[3]

(Accepted for publication July 1, 1951)

Verticillium wilt is the most important fungus disease of tomato in Utah. It occurs every year in the cool coastal sections of California (5) and occasionally in most of the northern States. The fungus *Verticillium albo-atrum* Reinke & Berthold causes the disease.

Resistance in the tomato to a number of pathogens has been the subject of several recent genetic studies. Resistance to each of the pathogens, *Septoria lycopersici* Speg., *Fusarium oxysporum* f. *lycopersici* Snyder & Hansen, and *Stemphylium solani* Weber and to the spotted wilt virus was found to be based on a single dominant factor difference (1, 2, 3, 4).

MATERIALS AND METHODS

Parental material and breeding procedure. In 1932, the late H. L. Blood received from J. W. Lesley, California Agricultural Experiment Station, a small-fruited, wilt-resistant strain of tomato called Peru Wild. Blood gave it Utah accession number 665. He crossed Number 665 with Century and selected in the succeeding generations for large fruit and resistance to *Verticillium albo-atrum*. One selection from this cross was given the number W6. This strain is wilt-resistant and has large, many-celled fruits. It was used as one parent and Moscow, a good commercial variety although susceptible, was used as the other parent.

In the spring of 1949 reciprocal crosses of resistant × susceptible and susceptible × resistant tomatoes were made in the field. Seed of the F_1 and the backcross was grown in the greenhouse during the winter of 1949–1950. Seed for all succeeding generations was produced in the greenhouse by either selfing or crossing.

Source of the pathogen and preparation of fungus inoculum. Because isolates of *V. albo-atrum* from tomato and eggplant in Utah and from tomato in California were found in tests to be equally pathogenic, cultures from both States were used. These were grown on potato-dextrose agar in Petri dishes for 7–14 days and were then macerated in a Waring Blendor. Sufficient water was added to give the mixture a thin soupy consistency.

[1] A thesis submitted by the senior author to the Graduate School of the Utah State Agricultural College, in partial fulfillment of the requirements for the degree of Master of Science in botany, with additional data supplied by the junior authors.

This study grew out of a project to develop wilt-resistant tomatoes. Until 1948 the project was directed by Dr. H. L. Blood, plant pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture. Deceased 1948.

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Inoculation and planting of seedlings. Seed was planted in sand in greenhouse flats. When the seedlings were 1–1½ in. tall and before the first true leaves appeared, the plants were dug and the roots dipped into the fungus inoculum. Then the plants were transplanted to pasteurized sandy loam in greenhouse benches, 2 in. apart in rows 2 in. apart (Fig. 1, A).

FIG. 1. A. Tomato seedlings growing in soil on greenhouse bench, 5 days after inoculation with *Verticillium albo-atrum*. B. Healthy seedling 1 week after inoculation. C. Seedling with wilted cotyledons, first symptom of the disease. D. Plants 1 month after inoculation. The stunted plants in the front row are of the susceptible variety Moscow. Plants in the back row are of a backcross population and are segregating 1 resistant to 1 susceptible.

Classification of resistant and susceptible plants. If soil temperatures were maintained between 21° and 25° C. and soil moisture was kept slightly above optimum for growth of tomatoes (Fig. 1, B, C), cotyledons of susceptible plants wilted 1 week after inoculation and then died; plants were stunted and many of them died (Fig. 1, D). If soil temperature was lower than 20° C. for as long as 8 hr. each day during the first week after inoculation, susceptible plants did not wilt, but most of them were stunted within 3 weeks of the time of inoculation. Except for occasional plants which failed to grow properly, resistant plants did not wilt and were not stunted. (See large plants in figure 1, D.)

Plants included in various tests. Results of three tests are reported in this paper. Resistant and susceptible parents and the F_1, F_2, and backcross progenies were included in test 1. The F_3 families and the resistant and susceptible parents were included in test 2. The F_3 families of the first backcross, F_2's of the second backcross, F_1's of the third backcross, and the susceptible parent were included in test 3.

RESULTS

The data obtained have been interpreted as supporting the theory that resistance is based on a single dominant factor difference. In this discussion resistance is denoted by *Ve* and its recessive allele by *ve*.

Behavior of parents. Of 192 plants of the resistant parent (W6), 188 were classified as resistant (Table 1). Four plants of the resistant parent

TABLE 1.—*Verticillium wilt reaction of the parental types, F_1, F_2, and backcross progenies*

Material tested	Suggested genetic symbols	Number of plants			P value
		Total	Not stunted	Stunted	
Parents					
Resistant parent	*Ve Ve*	192	188	4	
Susceptible parent	*ve ve*	192	18	174	
F_1 generation					
Susc. × res.	*Ve ve*	156	146	10	
Res. × susc.	*Ve ve*	166	163	3	
F_2 generation					
Susc. × res.	*Ve Ve, Ve ve,* & *ve ve*	328	244	84	0.8–0.9
Res. × susc.	*Ve Ve, Ve ve,* & *ve ve*	328	236	92	0.2–0.3
F_1 backcross					
Susc. × (susc. × res. F_1)	*Ve ve* & *ve ve*	164	85	79	0.5–0.7

were stunted; however, it was assumed that *Verticillium albo-atrum* did not cause the stunting, because stunting was observed occasionally in noninoculated resistant plants growing in pasteurized soil. The exact cause of this stunting was not determined.

Of 561 plants of the susceptible parent (Moscow), 542 were classified as susceptible. The 19 plants that were not stunted were assumed to be escapees (Tables 1 and 3). All progenies of such escapees were susceptible, which was evidence that the assumption was correct.

Behavior of the F_1. Of the 322 F_1 plants of the reciprocal crosses, 309 were not stunted and 13 were stunted (Table 1). As with the resistant parent (W6), it is believed that causes other than susceptibility to the fungus were responsible for this stunting.

Behavior of the F_2. Second-generation hybrids were represented by 656 plants, half from the cross of resistant × susceptible and half from the reciprocal cross. Of the resistant × susceptible plants, 236 were resistant and 92 susceptible. In the susceptible × resistant cross 244 plants were resistant

and 84 susceptible. Both populations agree satisfactorily with the expected 3:1 ratio, as indicated by the P values (Table 1).

Behavior of the backcross. The backcross, susceptible × (susceptible × resistant F_1), was represented by 164 plants, of which 85 were resistant and 79 susceptible. The P value for goodness of fit to the expected 1:1 ratio shows satisfactory agreement.

Behavior of the F_3. Inasmuch as some susceptible plants recover when placed under different environment, seed was obtained from nine stunted F_2 plants. In tests of the F_3 families[4] obtained from stunted plants, eight families, numbering 418 plants, were susceptible and one segregated 3 resistant to 1 susceptible, indicating that one plant was improperly classified in the F_2.

Of the families from 14 resistant F_2 plants of the cross resistant × susceptible, six were homozygous resistant and eight segregated 3 resistant to 1 susceptible (Table 2). Of the families from 20 resistant F_2 plants from

TABLE 2.—*Verticillium wilt reaction of F_3 families classified as resistant in the F_2*

Classification	Resistant *Ve Ve* × Susceptible *ve ve*	Susceptible *ve ve* × Resistant *Ve Ve*
Ve Ve	6 families (603 plants)	4 families (478 plants)
Ve ve or segregating	8 families (1,006 plants)	16 families (1,381 plants)
	P value, 0.3–0.5	P value, 0.2–0.3
Classification of individual plants in segregating families		
Ve Ve or *Ve ve*	770 plants	1,057 plants
ve ve	236 plants	324 plants
	P value,[a] 0.2–0.3	P value,[b] 0.1–0.2

[a] Range in P value for individual families was 0.05–0.7.
[b] Range in P value for individual families was 0.2–0.99.

the reciprocal cross, four were homozygous resistant and 16 segregated 3:1. P values for goodness of fit to the expected 2:1 ratio showed satisfactory agreement.

Behavior of backcross populations. The completed data of test 1 led to the conclusion that resistance was based on a single factor difference and therefore the backcross method could be used in developing resistant strains of any variety. Accordingly, resistant plants of the first backcross were selfed and crossed with the susceptible parent. Tests were conducted to determine the resistance of the resulting F_2's and backcrosses, and resistant plants were selected from both groups. The F_2's were selfed and the F_1's were selfed and backcrossed. Results obtained when progenies of these were tested for resistance (Table 3) support the evidence obtained in the previous tests.

Of the 29 F_3 families, seven (369 plants) were classified as homozygous resistant, and 22 segregated 3:1. The expected ratio of homozygous resistant to segregating families is 1:2. The observed ratio, 7:22, is well

[4] The term "family" refers to the F_3 progeny of an F_2 plant.

TABLE 3.—*Verticillium wilt reaction of backcross populations*

Lines tested	Number of lines		Segregating families			P value	Range in individual P values
	Resistant *Ve Ve*	Segregating	Total	Resistant or not stunted	Susceptible		
F_3 of first backcross[a] (Susc. × res.) × susc.	7	22	1,665	1,235	430	0.3–0.5	0.1 –0.99
F_2 of second backcross[b]	0	5	344	253	91	0.5–0.7	0.1 –0.9
F_1 of third backcross	0	4	330	164	166	0.9–0.95	0.05–0.7
Moscow (susceptible parent)			369	1	368		

[a] Obtained by selfing resistant plants of the first backcross and then selecting and selfing the resulting resistant plants.

[b] Obtained by selfing resistant plants of the second backcross.

within the error of sampling. The 22 individual segregating F_3 families conformed with the theoretical ratio of 3 resistant plants to 1 susceptible plant (Table 3).

Five F_2 families of the second backcross segregated 3 : 1. There were 253 resistant and 91 susceptible—a deviation of five from the theoretical number. The P values for the individual families and total plants indicate satisfactory agreement with the 3 : 1 ratio.

Plants in the four lines of the third backcross segregated 164 resistant to 166 susceptible—a deviation of one from the expected 1 : 1 ratio.

SUMMARY

Inheritance studies were made on 322 F_1 plants, 656 F_2 plants, 42 F_3 families which involved 3,886 plants, 164 first backcross plants, and 2,708 plants in continued backcross populations derived from the first backcross.

The data obtained in this study indicate that resistance to *Verticillium albo-atrum* in W6 differs from that in Moscow by a single dominant Mendelian factor. The factor symbol has been assigned for W6 as *Ve Ve* and for Moscow as *ve ve*.

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THE SELECTIVE ACTION OF PENTACHLOROPHENOXYACETIC ACID AGAINST STREPTOMYCES SCABIES (THAXT.) WAKSMAN & HENRICI IN CULTURE MEDIA

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(Accepted for publication July 2, 1951)

Relatively low concentrations of 2,4-D (2,4-dichlorophenoxyacetic acid) and other synthetic plant hormones have selective fungistatic and bacteriostatic activity as well as selective herbicidal properties. The selective systemic fungicidal effects of 2,4,6-trichlorophenoxyacetic acid, of pentachlorophenoxyacetic acid, pentachlorophenoxy*iso*butyric acid, and (2-naphthoxy)-phenylacetic acid against infection of broad bean (*Vicia faba*) by *Botrytis cinerea* have recently been reported by Crowdy (2). None of these chemicals at the concentrations used caused injury to the bean plants. Applications were carried out on young seedlings grown for two or three weeks with their roots immersed in solutions containing 10 p.p.m. of the acids. After treatment plants were sprayed with a suspension of spores and kept in a saturated atmosphere until symptoms developed; the damage to each leaf was then assessed on an arbitrary scale.

Michaelson (5) reported the fungistatic properties of several synthetic plant hormones, including pentachlorophenoxyacetic acid, against four races of Streptomyces. Carlyle and Thorpe (1) and Payne and Fults (6) found that 2,4-D, when mixed with the soil in amounts above 0.075 lb. per acre, interfered with the nodulation of beans. Stevenson and Mitchell (8) found that open test tubes of potato-dextrose agar containing 0.10 per cent 2,4-D remained sterile for 30 days and that 0.08 per cent 2,4-D inhibited *Bacillus subtilis* (Ehrenberg) Cohn, but did not affect *Penicillium notatum* Westling. Worth and McCabe (9) showed that aerobic bacteria are inhibited by 2,4-D at concentrations as low as 20 p.p.m. at pH 7.4. Richards (7) studied the effect of various growth-regulating substances on four species of fungi. As a rule they were stimulated in the 10^{-3} to 10^{-4} M range and inhibited between 10^{-3} and 10^{-1} M.

The objectives of the investigations reported in the present paper were 1) to study the direct effects of pentachlorophenoxyacetic acid on *Streptomyces scabies* (Thaxter) Waksman & Henrici No. 128, 2) to determine whether these effects were fungistatic or fungicidal, and 3) to determine the selective effect of pentachlorophenoxyacetic acid against *Streptomyces scabies* No. 128 when present in the same culture plate with *Escherichia coli* (Migula) Castellani & Chalmers, *Micrococcus pyogenes* var. *aureus* (Rasen-

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bach) Zopf., and two forms of *Penicillium notatum* Westling. Form numbers were 54 and 90.[2]

MATERIALS AND METHODS

The culture medium used was a modified potato-dextrose agar prepared as described by Michaelson (4, 5). The race of *Streptomyces scabies* used was isolated from soil collected in the Buckhorn Canyon area, near Fort Collins, Colorado, by L. A. Schaal, Bureau of Plant Industry, U. S. Department of Agriculture. It was selected because of its marked antibiosis to many fungi. The two forms of *Penicillium notatum* Westling were isolated from soil samples collected at the U. S. Department of Agriculture Potato Experiment Station near Greeley, Colorado, and at the Larkin farm near Eaton, Colorado. The *Micrococcus pyogenes* var. *aureus* and the *Escherichia coli* were supplied by the Department of Bacteriology, Colorado A & M College.

The study of the fungistatic and fungicidal effects of pentachlorophenoxyacetic acid was limited to *Streptomyces scabies* No. 128. This was done by first inoculating a series of potato-dextrose agar plates containing 0, 4, 8, 16, 32, 125, 250, 500, 1000, and 2000 p.p.m. of pentachlorophenoxyacetic acid with the test organism. Five weeks after inoculation, a second set of potato-dextrose agar plates containing no chemical were inoculated from each of the first tests. If the organism grew in the second set of plates the action was considered fungistatic at that concentration. Failure to grow was taken to indicate fungicidal action.

The selective effect of pentachlorophenoxyacetic acid against *Streptomyces scabies* No. 128, when grown in the same plates with the two forms of *Penicillium* and the two bacteria, was studied by a "cross" technique. In this technique the plates were first inoculated with *Streptomyces scabies* No. 128 in the form of a cross (+). Two days after inoculation and incubation at 22° C., each of the axes of the cross was inoculated with the *E. coli*, the *M. pyogenes* var. *aureus*, or one of the two forms of *P. notatum*. This technique was used on a series of modified potato-dextrose plates containing 0, 4, 8, 16, 32, 64, 125, 250, 500, 1000, and 2000 p.p.m. of pentachlorophenoxyacetic acid. After inoculation all plates were kept at room temperature (average 22° C.) for 5 weeks and observations and measurements were then made.

RESULTS AND DISCUSSION

Study of the direct effects of pentachlorophenoxyacetic acid. Five weeks after inoculation, there were no visible effects of the chemical on *Streptomyces scabies* No. 128 in the range of 0–16 p.p.m. At 32 and 64 p.p.m. growth was limited, and at 125 p.p.m. and above growth was inhibited. Figure 1 illustrates the effects of the chemical one week after inoculation;

[2] Isolated from soil samples collected near Greeley, Colorado, and identified by L. W. Durrell, Botany and Plant Pathology Department, Colorado A & M College, Fort Collins, Colorado.

growth was limited at 8 p.p.m. (Petri plate 2) and eliminated at all higher concentrations.

Similar tests were made with the two forms of *P. notatum* and the two bacteria. All four organisms attained their greatest growth in the control plates and were inhibited to increasing degrees by the increasing concentrations of the pentachlorophenoxyacetic acid. Figure 2 illustrates the direct effects on *P. notatum*.

FIG. 1. The effect of pentachlorophenoxyacetic acid on race No. 128 of *Streptomyces scabies*. Plates 0 to 10 contained 0, 4, 8, 16, 32, 64, 125, 250, 500, 1000 and 2000 p.p.m. respectively of pentachlorophenoxyacetic acid in modified potato-dextrose agar. Photographed one week after plates were inoculated.

Determination of fungicidal-fungistatic effects on Streptomyces scabies No. 128. The cultures on the plates containing 125, 250, and 500 p.p.m. of pentachlorophenoxyacetic acid grew when the *Streptomyces scabies* No. 128 was reinoculated onto modified potato-dextrose agar plates containing no pentachlorophenoxyacetic acid. Transfers from the 1000- and 2000-p.p.m. plates failed to grow. These results indicate that the action of pentachlorophenoxyacetic acid against *Streptomyces scabies* No. 128 is fungistatic in the range between 32 and 500 p.p.m. and is probably fungicidal at 1000 p.p.m. and above.

Selective action of the acid against Streptomyces scabies No. 128 in mixed culture. Streptomyces scabies No. 128 was grown in mixed culture with *E. coli*, *M. pyogenes* var. *aureus*, and two forms of *P. notatum*, on potato-dextrose agar containing from 0 to 2000 parts of the acid. The interacting effects of pentachlorophenoxyacetic acid plus the antibiotic effects of

the *Streptomyces scabies* No. 128 are evident in the data in table 2. Average colony diameters of the *E. coli*, *M. pyogenes* var. *aureus*, and the two forms of *P. notatum* were taken as a measure of the interacting effects. All four organisms were more tolerant of the pentachlorophenoxyacetic acid than was *Streptomyces scabies* No. 128. Average colony size of all four organisms was increased at certain concentrations and decreased at others. This was interpreted to mean that as the growth and antibiotic action of the *Streptomyces scabies* was inhibited, the four associated organisms increased their growth. At high levels of the chemical all organisms were inhibited.

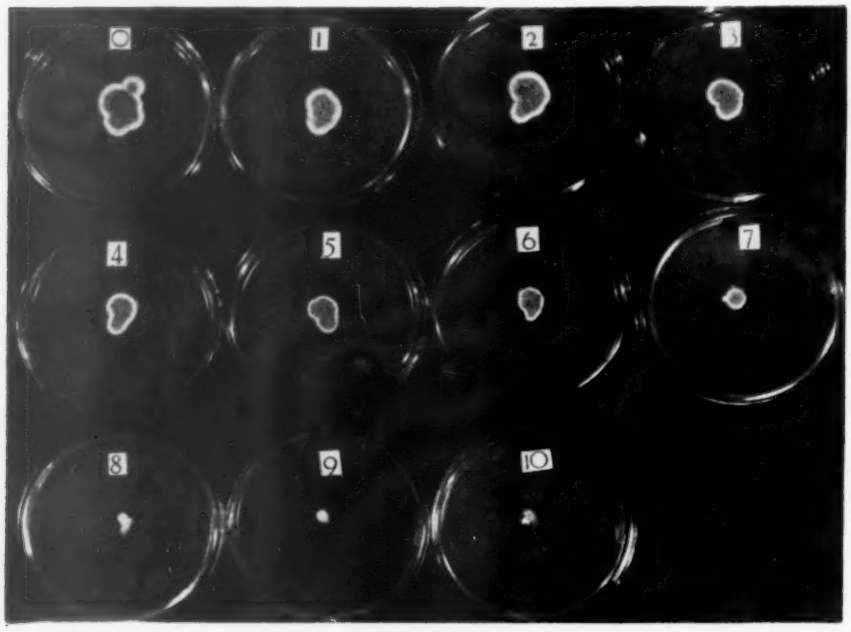

FIG. 2. The effect of pentachlorophenoxyacetic acid on a strain of *Penicillium notatum*. Plates 0 to 10 contained 0, 4, 8, 16, 32, 64, 125, 250, 500, 1000 and 2000 p.p.m. of pentachlorophenoxyacetic acid in modified potato-dextrose agar.

E. coli increased in colony size between 8 p.p.m. and 250 p.p.m. and thereafter, i.e., at 500, 1000, and 2000 p.p.m., decreased. At 2000 p.p.m., colony diameters were only one-tenth of the control diameters. The colony diameters of *M. pyogenes* increased in the range between 8 and 64 p.p.m. and then decreased to one-half the control at 250 p.p.m. At 500 p.pm. and above there was no growth. The two forms of *P. notatum* reacted in a similar manner. In both cases growth was increased in the range 16–64 p.p.m. In both forms growth was greater than the controls up to a concentration of 500 p.p.m. The growth of both organisms was inhibited at 2000 p.p.m.

The effect of pentachlorophenoxyacetic acid on the *Streptomyces scabies* in these tests was similar to the direct effects: very little visible effect at 16 p.p.m. and less, limited growth at 32 and 64 p.p.m., and no growth at 125 p.p.m.

Unpublished work of the senior author (3) on the use of pentachlorophenoxyacetic acid as a soil treatment for the control of the potato scab disease has indicated that this chemical inhibits or prevents the growth of the causal agent, *Streptomyces scabies.* Rates tested were 1 and 10 lb. per acre. In these studies soil treatment produced a significant reduction in the percentage of area covered by scab pustules on Bliss Triumph potatoes. Treated plants produced a significantly higher percentage of scab-free tubers. Scab pustules that did form were shallower than in the controls.

These results emphasize the importance of the selective action of pentachlorophenoxyacetic acid in culture media against *Streptomyces scabies.* An ideal soil fungicide probably is one which is selective against specific pathogens, because a soil without fungi or bacteria is a dead soil.

TABLE 1.—*The effect of pentachlorophenoxyacetic acid on average colony diameter of Escherichia coli, Micrococcus pyogenes var. aureus, and two forms of Penicillium notatum grown on modified potato-dextrose agar in the presence of Streptomyces scabies No. 128; 5-week-old cultures*

Chemical concentrations (p.p.m.)	Average colony diameters (in mm.)			
	E. coli	*P. notatum* No. 90	*M. pyogenes* var. *aureus*	*P. notatum* No. 54
0	10[a]	10	2	5
4	10	10	2	5
8	12	10	4	5
16	13	18	5	20
32	14	18	6	22
64	15	22	8	25
125	25	21	2	24
250	27	20	1	19
500	23	15	0	10
1000	20	1	0	10
2000	1	0	0	0

[a] Values shown to nearest whole mm. Each value is an average of five replications.

SUMMARY

Growth of *Streptomyces scabies* No. 128 in modified potato-dextrose agar medium was limited by pentachlorophenoxyacetic acid at concentrations of 32 and 64 p.p.m., and was inhibited at 125 p.p.m. The action of the chemical was considered fungistatic between 32 and 500 p.p.m. and fungicidal at 1000 and 2000 p.p.m.

Escherichia coli, Micrococcus pyogenes var. *aureus,* and two forms of *Penicillium notatum* were more tolerant to pentachlorophenoxyacetic acid than was *Streptomyces scabies* No. 128, thus indicating that the chemical has a selective effect.

Preliminary field tests in the northern Colorado potato-growing area indicate that pentachlorophenoxyacetic acid used as a soil fungicide reduces potato scab disease caused by *Streptomyces scabies*.

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ANTHRACNOSE OF DIGITALIS

C. A. Thomas

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A few hundred acres of digitalis for medicinal purposes are cultivated in the United States. The acreage is mostly under contract and varies in size from year to year. This limited production is mainly in Pennsylvania, Wisconsin, and the New England States. *Digitalis purpurea* L., the principal species grown, has not been severely affected with anthracnose. Cultivation of a related species, *D. lanata* Ehrh., has been attempted in recent years. This species was known to be affected with anthracnose in Pennsylvania in 1946. In 1948, the disease caused almost complete loss of plantings near Reading, Pennsylvania.

Anthracnose of digitalis has been reported previously in this country by Osmun (7) in Massachusetts in 1915 and by Clinton (3) in Connecticut in 1934. The disease has occurred also in Europe (2, 5, 6, 8) and Asia (4). *D. purpurea, D. ferruginea* L., and *D. lanata* were mentioned as suscepts in the above reports.

SYMPTOMS AND LOSSES

Symptoms of the disease in Maryland and Pennsylvania are similar to those described by Unamuno (8) and Goto (4). Purplish-brown, circular or angular spots, 1 to 4 mm. in diameter, are produced on the leaf surfaces; small, sunken, fusiform lesions occur on veins and petioles. Numerous dark brown setae are produced in the black, pulvinate acervuli. The pathogen, known to be seed-borne, fruits on damped-off seedlings and is easily spread in the seed bed. Lesions may not appear on the first true leaves of seedlings grown in outdoor seed beds before they are transplanted to the field. Infection becomes general and severe by late summer or early fall and severely affected plants are killed before the herb is ready to be harvested. Affected plants that are not killed are stunted, with a resulting loss in yield.

The effect of the disease on total cardiac glycoside content of the herb was determined by assaying leaf samples from healthy and diseased plants using a colorimetric method described by Bell and Krantz (1). The glycoside content of diseased leaves was not significantly lower than that of healthy leaves.

THE PATHOGEN

The pathogen was isolated easily from affected leaves of *D. lanata* and *D. purpurea* by tissue culture. Single-spore isolates from these two suscepts were identical in cultural characteristics and pathogenicity and they agreed with Laubert's description of *Colletotrichum fuscum* Laub. (5).

The rapid development of the disease in seed beds indicated that infested seed was possibly the primary source of inoculum. Numerous lots

of *D. lanata* and *D. purpurea* seed from Maryland, Pennsylvania, and Wisconsin were studied. It was difficult to identify and isolate the pathogen directly from seed or seed washings. A simple method of determining the presence of the organism on seed consisted of sowing a few hundred seeds in a greenhouse flat and observing the development of the disease over a period of 12 weeks. It was found that most lots of *D. lanata* seed from the three locations were infested. None of the lots of *D. purpurea* were infested. *D. purpurea* readily developed anthracnose, however, when seed was artificially infested.

TEMPERATURE

The relation of temperature to mycelial growth of the organism in culture was determined. On potato-dextrose agar, pH 5.6, in Petri dishes, the average diameter of the colony after 120 hr. was 4 mm. at 7.5° C., 21 mm. at 15°, 33 mm. at 20°, 37 mm. at 22.5°, 41 mm. at 25°, 27 mm. at 27.5°, and 18 mm. at 30°. The optimum temperature for growth, therefore, is 25° C. and the organism appears to be slightly less tolerant of temperatures above 25° than of lower temperatures.

The effect of temperature on disease development was studied also. Three-month-old plants of *D. lanata* were inoculated by atomizing with a spore suspension. Inoculated plants were placed in a greenhouse moist chamber for 48 hr. and then moved to constant-temperature chambers maintained at 15° C., 20°, 25°, and 30°. Light was provided by fluorescent bulbs. The disease developed most rapidly and was most severe at 25° and 30° C. with little difference noted between the two temperatures. Development was somewhat slower at 20° and greatly retarded at 15°.

CONTROL

Plantings of five available species of digitalis have been made at Beltsville, Maryland, during the last few years in connection with a program for improvement of yield and quality. Seed lots from several different sources were planted. Certain plantings of susceptible species often were free of anthracnose and it was found later that these plantings were started from noninfested seed. It thus appeared that the use of such seed would result in good control.

Naturally infested seed of *D. lanata* was subjected to various seed treatments to determine whether or not the organism on the seed could be completely controlled. Treated seeds were planted in greenhouse flats and notes on disease development were made over a period of 12 weeks. Arasan (50 per cent tetramethylthiuramdisulfide), Semesan (30 per cent hydroxymercurichlorophenol), and Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide) were applied full strength and diluted with one, two, three, and four parts talc. All applications were made at the maximum dust-holding capacity of the seed. All treatments were extremely injurious; either the seed failed to germinate, or the young seedlings were soon killed.

Talc alone was not injurious but it did not result in control of anthracnose. Seed was treated also with hot water. After soaking in cold water for 4 hr. and tempering at 50° C. for 1 min., seed was treated at 55° for 1, 5, 10, 15, 20, 25, and 30 min. Treatment for 15 min. or longer gave complete control of the organism. Marked reduction in germination usually resulted from treatments longer than 15 min. but there was considerable variation among different seed lots in the amount of injury caused by the hot-water treatment. The above experiments were repeated four times. Good stands of disease-free seedlings were obtained consistently with the 15-min. hot-water treatment. Seedlings grown in greenhouse flats from comparable untreated seed developed anthracnose within 4 weeks, and usually all the seedlings in such flats were dead within 8 weeks.

Isolated single-row plantings were made in the field at Beltsville, Maryland, in 1949 with *D. lanata* and *D. ferruginea* seedlings from infested and noninfested seed. The use of noninfested seed resulted in complete freedom from anthracnose. In 1950, four isolated plots in Maryland and Pennsylvania, each approximately 1/50 acre in size, were planted with seedlings of *D. lanata* from untreated, naturally infested seed. An additional four plots were planted with seedlings from the same seed treated with hot water. All plants from the treated seed remained free of anthracnose throughout the season, whereas the plants from the untreated seed were severely affected.

SPECIES SUSCEPTIBILITY

The relative susceptibility of five species of digitalis was determined in field plots in 1949 and 1950 under conditions of natural infection at Beltsville, Maryland. All species tested were susceptible. The amount and severity of infection indicated that *D. sibirica* Lindl. and *D. purpurea* were the most resistant, *D. ambigua* Murr. intermediate, and *D. ferruginea* and *D. lanata* the most susceptible.

The susceptibility of the above species was determined also in the greenhouse. Two-month-old plants of each of the five species were inoculated by atomizing with a spore suspension and incubating in a moist chamber for 48 hr. Symptoms developed after 8–10 days. In most tests the relative susceptibility of the species was the same as in the field plots. Heavy infection was obtained more readily on the susceptible species than on the resistant species. Whole plants or leaves of resistant species were severely injured, however, in cases where heavy infection was obtained.

SUMMARY

Anthracnose, caused by *Colletotrichum fuscum* Laub., has caused considerable damage to *Digitalis lanata* in commercial plantings. The disease was avoided by using disease-free seed. A hot-water treatment, 55° C. for 15 min., was feasible for controlling the organism on naturally infested seed. Temperature studies showed that the disease developed best at 25°

and 30° C. Considerable difference in susceptibility was noted among five species of digitalis.

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THE OVERWINTERING OF THE CUCUMBER MOSAIC VIRUS[1]

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(Accepted for publication August 8, 1951)

The cucumber mosaic disease affects a number of crop and ornamental plants (12, 16) both in the field and in greenhouses, and control measures are often sought. The virus is short-lived under natural conditions in dead tissues and hence requires a perennial host that survives winter weather, or between crop seasons, in order for the virus itself to survive and initiate new infection on annual plants. Seed transmission of the virus is apparently not involved in control problems, but the virus is regularly transmitted by certain species of aphids. In the absence of suitable resistant varieties control measures, therefore, seemed to be dependent upon the elimination of the vector or the known overwintering hosts. However, the virus has a wide experimental host range, and in addition a number of other species may be suspected of harboring the virus. The common milkweed (*Asclepias syriaca* L.) is widely distributed in Wisconsin and other northern States where it has been regarded as the most important source of overwintering virus. Because of some absence of observed correlation between occurrences of the disease on crops and on milkweed, disease control by eradication of this weed has lacked certain confirmation. An obvious problem in this connection was to discover other probable overwintering hosts and to determine the relation of the occurrence of natural hosts to crop infection. This challenge became the chief purpose of the present investigation.[3] A preliminary abstract of the results was presented in 1950 (8).

EARLIER LITERATURE

Although the known host-range of the cucumber mosaic virus had reached considerable proportions in recent years (12), milkweed appeared to be the chief perennial weed host in the north-central States. Its eradication, along with other known hosts, in and for a distance of 50 yards around cucumber fields as first recommended by Doolittle (4) and Doolittle and Walker (6) has been accepted as a standard measure for controlling the disease. In other areas it was recognized that pokeweed (*Phytolacca decandra* L.) and Wandering Jew (*Commelina nudiflora* L.) might be concerned (7, 16). Meanwhile new "strains" of the virus were being reported from various parts of the country and this contributed to uncertainty as to the host plants actually involved in overwintering in some regions. By 1950 the list of known host plants had reached approximately 200 and fully 30 strains (often isolates only) of the virus had been reported. During this

[1] This research was supported in part by grants from the Wisconsin Alumni Research Foundation.

[2] Now at Plant Pathology Department, Lingnan University, Canton, China.

[3] The writers are indebted to Dr. R. W. Fulton for assistance throughout this investigation.

period, however, the characteristics of the cucumber mosaic virus were being more exactly defined on the basis of properties, cross-protection tests, insect relations, etc. This has made possible more reliable investigations and interpretation of results. As a result the number of probable strains has been lowered by the necessary conclusion that some earlier reports were not sufficiently complete to warrant definite distinction. It is, nevertheless, obvious that the problem of overwintering of the virus is greatly complicated by the possible existence of important unknown hosts as well as unusual virus strains.

MATERIALS AND METHODS

The present study is based largely on field collections of material made during the growing season in Wisconsin. This material consisted mostly of leaves showing signs of virus infection, but often included apparently healthy plant materials suspected of being masked carriers. These field collections were made over an area of 17 counties, but particular attention was given to areas around commercial cucumber and tobacco fields and the vicinity of home gardens.

Ordinary tobacco (*Nicotiana tabacum*) is a reliable host plant for quick recognition of cucumber mosaic virus symptoms, as well as for maintaining the virus in culture. Consequently all collections were first inoculated to this host in the greenhouse. The positive determination or separation of the virus proceeded from this host to other differential hosts and to property studies, etc., until identified. For example, by the use of *N. glutinosa* or the hybrid host (*N. tabacum* × *N. glutinosa*) the presence of the tobacco mosaic virus was recognized on the inoculated leaves by the necrotic reaction, and the cucumber mosaic virus, if present in mixture, could be isolated from the younger, noninoculated leaves. Infection on cucumber, together with the symptom expression on tobacco, was usually sufficient to establish that the cucumber mosaic virus was involved. Property studies, host range, insect relations, and cross-protection tests were resorted to in specific instances with aberrant strains.

EXPERIMENTAL RESULTS

In a search for the cucumber mosaic virus as it occurs in nature, it may be taken for granted that neither the host involved nor the symptom expression is a reliable criterion as to the virus concerned. In many early reports of virus infection, determinations further than field inspection were not made. Consequently, some of the reported occurrences of cucumber mosaic in nature are in need of verification and this phase was undertaken as an important part of the present investigation. The present survey of wild plants as well as field crops was made chiefly during the growing seasons of 1948 and 1949 within a radius of 100 miles of Madison. From about 160 species of annual, perennial, and biennial plants, 2153 samples were collected. Of these samples, 16.7 per cent (360 collections) were infected with

the ordinary cucumber mosaic virus or strains of this virus. This relatively low frequency of the virus in plants suspected of being infected is important and significant.

Among 41 hosts previously reported as naturally infected, 17 species were verified, including 10 annuals and seven perennials (Table 1). Twenty-four reported hosts were not found infected in the present survey. However, only 115 collections represent the negative results with the following species: aster (*Aster* sp.), bean (*Phaseolus vulgaris*), beet (*Beta vulgaris*), carrot (*Daucus carota*), celery (*Apium graveolens*), common chickweed (*Stellaria media*), chrysanthemum (*Chrysanthemum* sp.), eggplant (*Solanum melongena*), geranium (*Geranium* sp.), lettuce (*Lactuca sativus*), pansy (*Viola tricolor*), pea (*Pisum sativum*), pigweed (*Amaranthus retroflexus*), ragweed (*Ambrosia elatior*), scabiosa (*Scabiosa* sp.), sea-holly

TABLE 1.—*Relative frequency in Wisconsin of cucumber mosaic infection on previously reported annual and perennial host plants*

Host	Number of areas sampled—infested	Number of plants tested—infected	Percentage of plants infected
Annuals:			
Cucumber	47—29	83—56	67
Marigold	3—3	28—6	22
Muskmelon	3—3	8—6	75
Pepper	10—3	16—5	31
Petunia	20—11	41—19	46
Snapdragon	4—2	15—6	40
Squash	7—5	20—11	55
Tobacco	61—12	205—39	19
Tomato	23—16	130—43	33
Zinnia	18—11	36—21	58
Perennials:			
Catnip	25—3	79—5	6
Columbine	7—2	11—2	18
Larkspur	8—2	37—3	8
Milkweed	57—7	219—19	9
Petunia	4—1	6—2	33
Pokeweed	1—1	17—13	76
Virginia cowslip	1—1	5—1	20

(*Eryngium* sp.), shepherds-purse (*Capsella bursa-pastoris*), spinach (*Spinacia oleracea*), sweet potato (*Ipomoea batatas*), sweet corn (*Zea mays*), tulip (*Tulipa* sp.), watercress (*Nasturtium aquaticum*), wild cucumber (*Echinocystis lobata*), and wild ground cherry (*Physalis* sp.). Failure to find these plants infected with the virus is taken to indicate only that they are not commonly infected in the area surveyed.

New host plants in nature. Similar trials with other species collected from the field have yielded the cucumber mosaic virus in varying percentages from 11 species previously unreported as hosts in nature (Table 2). These field collections should not be confused with host ranges established by laboratory tests. Only two or three of these species are sufficiently common in the State to warrant consideration as likely sources of infection.

Perennial phlox (*Phlox drummondii* Hook.) is a common ornamental plant in Wisconsin. It was often heavily infested with aphids in the epidemic year of 1948, but was practically free from aphids in 1949. Wellman (15) reported that phlox in Florida was not infected with southern celery mosaic virus. The symptoms of infected phlox plants were ordinarily difficult to recognize. Powdery mildew, insect injury, dry weather effects, and other virus diseases caused a variety of abnormalities on the plants. Phlox seedlings inoculated with various strains of cucumber mosaic virus in the greenhouse all had severe stunting of plants, chlorosis, and mild mosaic on leaves.

In 1948, cucumber mosaic virus was found in eight samples from 96 collections of phlox from the campus of the University of Wisconsin and home gardens in Madison. Subsequently, large-scale collections of perennial phlox were made in all localities visited. In the 2-year period, 30 of 252

TABLE 2.—*Species naturally infected with the cucumber mosaic virus in Wisconsin, not previously reported,*[a] *with data indicating frequency of infection*

New natural hosts	Number of plants	
	Tested	Infected
1. Common motherwort (*Leonurus cardiaca* L.)	75	18
2. Flowering spurge (*Euphorbia corollata* L.)	33	2
3. Garden-heliotrope (*Valeriana officinalis* L.)	28	15
4. Gladiolus (*Gladiolus* sp.)	1	1
5. Greekvalerian (*Polemonium caeruleum* L.)	17	12
6. Phlox (*Phlox drummondii* Hook.)	252	30
7. Coneflower (*Rudbeckia* sp.)	2	2
8. White cockle (*Lychnis alba* Mill.)	138	13
9. Carpetweed[b] (*Mollugo verticillata* L.)	3	2
10. Gentian sage[b] (*Salvia patens* Cav.)	1	1
11. Spiderflower[b] (*Cleome spinosa* L.)	4	1

[a] Seven perennial and 10 annual species previously reported, excluding Wellman's celery mosaic.

[b] Annual species.

collections (12 per cent) distributed in 12 of 37 localities were infected with cucumber mosaic virus. The importance of perennial phlox as an overwintering host of the cucumber mosaic virus consequently may be greater than that of milkweed, catnip, or other plants. However, because of its distribution phlox is probably a more important overwintering host in home gardens than in commercial fields.

Although higher percentages of infection were found in other ornamental plants such as garden heliotrope (53 per cent) and Greekvalerian (71 per cent), these hosts are seldom planted in home gardens and so are less likely to be involved in overwintering than phlox.

White cockle (*Lychnis alba* Mill.), a common perennial weed, appears to be a favored host for aphids. Intensive surveys were made for this species in all localities visited. Thirteen collections of 138 yielded infected plants, of which seven were obtained from a large cucumber field. White-cockle

plants infected with cucumber mosaic in the field showed only a slight mottle or chlorosis. Artificial inoculations of seedlings in the greenhouse also produced a very mild mottle or chlorosis, and the susceptibility of the species to the virus reached only about 50 per cent by mechanical inoculation. The relation of white cockle to the overwintering problem remains to be determined.

The several other previously unreported species found infected in nature (Table 2) included common motherwort, flowering spurge, coneflower, spiderflower, carpetweed, gladiolus, and Gentian sage. These hosts, however, with the possible exception of motherwort, were neither sufficiently common in nature nor frequently enough infected to be considered as important overwintering hosts.

A number of other plant species suspected of being infected with cucumber mosaic virus have been tested; 427 collections of 63 determined species all gave negative results, and 101 collections of about 50 undetermined plant species were also free from the virus.

Distribution and frequency of infected hosts in nature. The results of the 2-year survey indicated that the distribution and frequency of infected hosts in nature are greatly affected by weather conditions in different seasons. In 1948 the weather was consistently warm in the spring and summer, the aphid population was very high, and infection, especially of cultivated annual plants, was very high. In spring, 1949, warm and cool weather alternated, the aphid population was rather low, and infected plants, especially cultivated annual plants, were very much fewer than in 1948. A similar situation, during this 2-year period, was reported to prevail in California (2).

In spite of the fact that the cucumber mosaic virus has been reported to have a wide host range in nature, the virus was found in the present survey on only 30 species—24 cultivated forms and 6 wild species. The latter were milkweed, catnip, white cockle, common motherwort, flowering spurge, and carpetweed.

Contrary to the reports by previous workers, milkweed (4, 6) was rarely found infected in the present survey. *Physalis* species reported as a wild host (14) was not found naturally infected, although it did harbor the tobacco mosaic virus. Except for occasional infected plants of white cockle, catnip, flowering spurge, and carpetweed in two commercial cucumber fields and two tobacco fields, all other infected wild hosts were in home gardens in or near villages. The frequency of infection of these perennial hosts was rather low (Table 1, 2) for the species commonly found in the State.

Although infected weed hosts were rare among cultivated crops, cucumber was frequently found infected with the cucumber mosaic virus in commercial fields (Table 1). It was not unusual to find one field heavily infected, whereas other fields only a few miles distant were only slightly infected or disease-free. Although tobacco is very susceptible to cucumber mosaic virus, the incidence of infection in the 2-year period was very low in

the field: only 13 infected of 80 samples from 60 localities, and 26 infected of 125 samples from the local experimental crop. In some years the cucumber mosaic virus is more widespread on tobacco in Wisconsin (10). Some large commercial tomato fields observed during this survey were generally free of the virus. In villages all susceptible species of crops and ornamental plants frequently had a very high percentage of infection. This was especially true with cucumber, tomato, petunia, garden heliotrope, and others. On the other hand, infection of perennial phlox, columbine, and larkspur was found only in gardens, and the percentage was low. These plants may be resistant to the disease, or they may may not be preferred food plants of the vectors.

It appeared from this survey that the distribution and frequency of infected plants as a whole were much higher in the cultivated species than in the wild species and higher in the annual species than in the perennial species. Van der Plank (13) points out that wild species and perennials which may have been exposed to virus diseases in the past are likely to be more resistant under natural conditions than crop plants. The results obtained in the present survey are in accordance with this idea.

Symptom variation on natural hosts. Some of the earlier conclusions on the overwintering hosts of the cucumber mosaic virus, such as milkweed (5, 6) and wild ground cherry (6, 14), were based chiefly on field symptoms. Our observations indicate that this method is not reliable or satisfactory for some hosts, particularly perennial hosts. This may be illustrated by the data on milkweed since an extensive survey was made for this plant. Of 42 collections of milkweed plants with conspicuous mosaiclike symptoms, only eight yielded cucumber mosaic virus; and of 94 collections with mild mosaiclike or chlorotic symptoms, only nine yielded the virus. On the other hand, one positive infection was obtained from a collection of symptomless plants. Considering all collections of milkweed plants showing symptoms, only 12.5 per cent yielded cucumber mosaic virus. From these results it might be supposed that the cucumber mosaic virus is not readily transmissible from milkweed to tobacco. This was not the case whenever known infected milkweed plants were used. Even where infected milkweed was stored in the refrigerator before inoculation at about 3° C. for 4 days, almost 100 per cent infection was secured in seven trials. The only logical conclusion from the data is that milkweed is not frequently infected with cucumber mosaic virus, especially around commercial fields.

Catnip was suggested by Muncie (11) in 1923 as a possible overwintering host of the cucumber mosaic virus. Doolittle and Walker (5, 6) reported observing mosaic catnip plants in Wisconsin, but the virus was apparently not positively identified. However, out of 79 collections made in this survey, five collections (three from a very large cucumber field and two from home gardens) were definitely infected with the cucumber mosaic virus. Catnip plants with striking mosaic or mottle symptoms were frequently infected, not with cucumber mosaic virus but with a ring-spot virus disease. Catnip

plants infected with cucumber mosaic virus had only mild mottle or mild chlorosis.

Although cucumber plants with mosaic symptoms were usually infected with cucumber mosaic virus, this cannot always be determined from observation. In four areas, severely diseased cucumber plants were infected with another virus, apparently tobacco ring spot. The field symptoms produced by this virus on cucumber are not easily distinguishable from those of common cucumber mosaic virus.

Symptomless plants are often not free from cucumber mosaic virus or other mechanically transmissible virus diseases. The percentage of infection obtained from symptomless plants, however, was in general much lower than from plants showing mild or doubtful symptoms. It is obvious that symptom expression in the field is not a reliable criterion for diagnosis of infection with the cucumber mosaic virus.

Infection of crop plants. Of the 25 commercial cucumber fields, representing about 600 acres, which were thoroughly surveyed during the two years (Table 3), 13 fields had a high percentage of plants infected with cucumber mosaic virus. Four other fields were extensively infected with an unidentified ring-spot virus. Eight fields appeared superficially to be free of mosaic. Twenty-one tobacco fields representing about 200 acres were surveyed in the same way. Samples of mosaic on tobacco suspected of being cucumber mosaic were also collected from an additional 40 fields, but the weed hosts in the vicinity were not determined. Cucumber mosaic was found in only two of 21 fields surveyed in 1948 and 1949, and the percentage of plants infected was low. Fifty-five home gardens in the tobacco-growing areas were surveyed, and 31 of these were found to have infected annual garden plants, especially cucumber. Evidently the proximity of other infected species does not normally cause epidemics of cucumber mosaic in tobacco fields in the area.

TABLE 3.—*Results of a survey for cucumber mosaic in perennial hosts in the vicinity of growing annuals susceptible to this virus*

Collection vicinity and number of areas	Numbers of perennials tested—infected			
	Milkweed	Physalis	Catnip	Phlox
Cucumber fields (25)	96—0	15—0	19—3	2—0
Tobacco fields (21)	34—0	4—0	14—1	9—2
Home gardens (55)	89—19	5—0	46—1	241—28

Annual plants are obviously of no importance in relaying the virus from season to season, and consequently play no part in the problems under consideration. The survey in Wisconsin revealed only five perennial wild hosts (milkweed, catnip, white cockle, flowering spurge, and common motherwort) and nine perennial and biennial ornametal hosts (columbine, larkspur, Vir-

ginia cowslip, perennial petunia, garden heliotrope, gladiolus, Greekvalerian, perennial phlox, and Wandering Jew).

It has been pointed out earlier that most of the wild and cultivated species infected with cucumber mosaic virus were in home gardens. Perennial wild hosts were rarely found infected in the commercial fields. Infected white cockle and catnip were found in only one commercial cucumber field, and two infected flowering-spurge plants in another field. Since the tests with flowering spurge yielded only a very few infections, this plant is accepted as an overwintering host of the virus, with reservation. In three tobacco fields only one catnip, one white cockle, and two perennial phlox were found infected with the virus. Since only suspected host plants were collected in most cases, the actual percentage of infected plants in the commercial fields is obviously very low.

Because milkweed, *Physalis*, and catnip have been most commonly suspected as the overwintering hosts of the virus, special attention was given to these hosts (Table 3). It is evident that except for an occasional infected plant the infected perennial hosts were not normally found infected in the commercial fields. In 25 diseased cucumber fields none of the milkweeds growing in or near the fields were found infected with the cucumber mosaic virus, nor with any other mechanically transmissible virus. In villages, however, seven home gardens had milkweeds infected with the cucumber mosaic virus. *Physalis* was never found infected with cucumber mosaic virus in any locality, although all suspected plants were collected and tested. Only three infected catnip plants were found in a cucumber field, one in a tobacco field, and one in a home garden. It is significant to note that crop plants were often found infected in many fields and home gardens in which there was no milkweed, *Physalis*, or catnip in or around the area.

In the present investigation perennial phlox (with 12 per cent of all plants tested found infected) was equally as important as milkweed (9 per cent infected) as an overwintering host of the cucumber mosaic virus (Table 3). The overwintering hosts, as a group, are evidently the only possible source of the virus after the winter season in the State. Yet it appears that the eradication of all perennial hosts surrounding commercial cucumber and tobacco fields is to be regarded as of doubtful value as a control measure.

DISCUSSION

The importance of an overwintering host of the cucumber mosaic virus may best be determined on the basis of its distribution and frequency in nature. Comparing infection found on 15 perennial and biennial plant hosts in Wisconsin, the data reveal no single overwintering host of the cucumber mosaic virus that can be held responsible. The perennial hosts as a group may be important in some areas. We have not been able to verify the field results obtained by Doolittle and Walker (5, 6) and Walker (14) some 25 years ago on common milkweed and perennial *Physalis* in Wisconsin.

Field observations and subsequent inoculation tests showed that the diseased appearance of milkweed and *Physalis* has little in common with infection with the cucumber mosaic virus. It is apparent that field observation alone is not reliable for judging infection of milkweed or other plants with cucumber mosaic virus. Inoculation tests are necessary to permit reliable conclusions being drawn from field material. This may explain why the results obtained in the present investigation do not conform to those reported by earlier workers (6, 14).

Although it is recognized that conclusions drawn from field observations over relatively short periods are not entirely reliable, such results may have a bearing on conclusions previously drawn from similar methods of approach. Since neither infected milkweed nor *Physalis* was found in or around fields that had been successively grown to cucumber for many years, and since the cucumber crop itself was heavily infected with this virus, the evidence that little or no relationship existed between these weeds and the disease in these fields was striking. In this connection, it is interesting to note that Cook (1) in 1937 reported that no natural hosts or any other source of infection could be found to account for the severe outbreak of cucumber mosaic in Puerto Rico. In England, on the other hand, bryony, a perennial cucurbit, is suspected of harboring the virus (9).

According to our present investigation the eradication of milkweed, *Physalis*, and other perennial hosts in or around commercial cucumber or tobacco fields does not appear to be justified. Epidemics of the cucumber mosaic disease apparently vary greatly from year to year. It is believed that this variation is due in part to a relationship between the environment and the aphid population as well as between the aphid population and the amount of the disease in nature. As is well known, aphids are very effective in spreading the disease for short distances. Whether the aphids distribute the virus over long distances, as would be necessary to account for some of our results, is a problem worthy of further consideration. Such insect transmission appears to be the chief unknown factor involved in attempting to control the disease by eradication of overwintering hosts. It should be noted that Doolittle (3) and Doolittle and Walker (5) recognized that beetles (*Diabrotica vittata* Fabr. and *D. 12-punctata*) may transmit the virus over a long distance, and this conclusion should also be investigated further.

SUMMARY

In a survey for the cucumber mosaic virus during the growing seasons of 1948 and 1949 in Wisconsin, 2153 specimens from 160 plant species were collected and tested. This survey included an area involving 17 counties and represented 635 collections of 48 annual species, and 1362 collections of 62 biennial and perennial species. Three hundred and sixty of these specimens yielded the cucumber mosaic virus. Fifteen annual species and 15 perennial or biennial species were infected with the cucumber mosaic virus.

Another 101 collections of about 50 undetermined plant species did not yield cucumber mosaic virus.

Among the perennial and biennial hosts previously reported in the literature, seven were confirmed in the present investigation. Eight new perennial hosts were found in nature. Of the 15 perennial hosts, only five were wild species: milkweed, catnip, common motherwort, flowering spurge, and white cockle. Several ornamental plants were found to be overwintering hosts, and of these perennial phlox appears to be more commonly infected, though less widely distributed than wild species such as milkweed.

The presence or absence of the disease in most cucumber and tobacco crops was not associated with the occurrence of infected milkweed, *Physalis*, catnip, or other known perennial host plants in the vicinity. Since nearly all infected overwintering hosts, including the wild perennial hosts, were found in home gardens in villages, it is doubtful that eradication of these wild hosts in or near commercial fields would be effective as a control measure against the disease.

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EFFECT OF 2,4-D ON SEEDLING BLIGHT OF WHEAT CAUSED BY HELMINTHOSPORIUM SATIVUM[1]

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Cereal crops are sometimes injured when sprayed with 2,4-D to control weeds. Moreover, certain plant pathogens, such as *Helminthosporium sativum*, are often associated with the injury. Therefore an attempt was made to ascertain whether 2,4-D predisposes wheat plants to attack by *H. sativum* or whether the effect of 2,4-D is on the pathogen.

Genetic variation in *H. sativum* has been induced in culture by changing the physical and chemical environmental conditions under which the organism is grown (2, 3, 6, 8). Investigations have been made in recent years on the effect of 2,4-D on the growth of various fungi on artificial media and on their pathogenicity (1, 9, 10); as yet no similar work has been done with this particular chemical on *H. sativum*. Hence the present work also includes a study of the effect of 2,4-D on the growth and genetic variation of *H. sativum*.

MATERIALS AND METHODS

Two monosporous isolates, races 1 and 2, of *H. sativum* P., K., & B. were used in the present studies. Both races are moderately pathogenic, produce spores abundantly, and are relatively stable on potato-dextrose agar.

Two varieties of wheat, Mida (C.I. 12008) and Carleton (C.I. 12064), were used in pathogenicity tests. Mida is moderately resistant to root rot; Carleton is moderately susceptible. The seeds for greenhouse tests were immersed in a solution of 1:1000 New Improved Ceresan for 20 min. and then thoroughly rinsed with water before drying.

Four formulations of 2,4-D were used: ammonium salt (ammonium 2,4-dichlorophenoxyacetate, acid equivalent 77.5 per cent); sodium salt (sodium 2,4-dichlorophenoxyacetate monohydrate, acid equivalent 80.5 per cent); amine (diethylamine salt, acid equivalent 39.7 per cent); and ethyl ester (ethyl ester dichlorophenoxyacetate, acid equivalent 35.2 per cent). The concentrations of 2,4-D in the medium ranged from 100 to 10,000 p.p.m. Detailed methods used in this investigation will be given under each particular experiment.

PATHOGENICITY OF HELMINTHOSPORIUM SATIVUM GROWN ON A 2,4-D MEDIUM

The experiments on the effect of 2,4-D on the pathogenicity of *H. sativum* were made in the greenhouse. Inoculum of race 1 was increased on

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potato-dextrose agar and on potato-dextrose agar containing 5,000 p.p.m. of the ammonium salt of 2,4-D, which was added to the medium after sterilization, before solidification. A suspension of spores and mycelium was made from each culture by scraping the fungus material off the agar and chopping it in a Waring blendor. Some of this suspension was added to each 4-in. pot of autoclaved soil. Seeds of Mida wheat were planted in triplicate pots in each treatment.

In this preliminary test the pathogenicity of race 1 of *H. sativum* on wheat was increased greatly after the fungus had been grown on a medium containing 2,4-D. There was poorer seedling emergence, heavier infection of roots, and lower green weight of plants in the pots inoculated with the fungus grown on potato-dextrose agar containing 2,4-D than in those inoculated with the fungus from ordinary potato-dextrose agar.

It was not clear whether the 2,4-D actually acted on the pathogenicity of the fungus or on the susceptibility of the host. In preparing the inoculum a very small amount of 2,4-D could have been transferred from the agar to the suspension of spores and mycelium; consequently 2,4-D might predispose the plants to attack by the pathogen and not increase the pathogenicity of the fungus at all. The experiment was repeated, therefore, with modifications. Inoculum was increased on potato-dextrose broth and on potato-dextrose broth containing 5,000 p.p.m. of the ammonium salt of 2,4-D. Part of the inoculum produced on the 2,4-D medium prior to application as suspension was washed in water; the remainder was applied without washing. (See table 1.)

TABLE 1.—*Effect of adding various types of inoculum of race 1 of Helminthosporium sativum to the soil in which Mida wheat seedlings were grown*

Source of inoculum	Percentage[a] of plants emerged	General vigor—degree of root infection	Green weight (gm.)
Control	96	Good—none	9.3
Potato-dextrose broth	72	Good—light	5.2
Potato-dextrose broth with 2,4-D, unwashed	56	Poor—heavy	2.0
Potato-dextrose broth with 2,4-D, washed	72	Fair—moderate	3.0

[a] Data are based on triplicated pots.

The infection of wheat plants by *H. sativum* again was more severe when the soil was inoculated with the fungus grown on the medium containing 2,4-D. However, infection was less with washed inoculum than with inoculum that was not washed. It is assumed that 2,4-D may have increased the severity of *H. sativum* on wheat by predisposing the wheat to attack by the pathogen rather than by increasing the pathogenicity of the

fungus. The washed inoculum caused a slightly heavier infection than the inoculum from ordinary potato-dextrose broth; perhaps the small amount of 2,4-D left in the washed inoculum was sufficient to predispose the plants.

EFFECT OF 2,4-D ON HOST-PARASITE RELATIONSHIP

In further laboratory and greenhouse experiments, seeds of Carleton durum were surface-disinfected with 1:1000 $HgCl_2$ and germinated on potato-dextrose agar in Petri dishes. A seedling 15–25 mm. long was inoculated with a single spore of race 1 of *H. sativum*, as described by Christensen and Schneider (4). Such inoculated plants were grown singly in test tubes of water agar containing 0, 1,000, 3,000, and 5,000 p.p.m. of ammonium salt of 2,4-D, which was added to the water agar after sterilization.

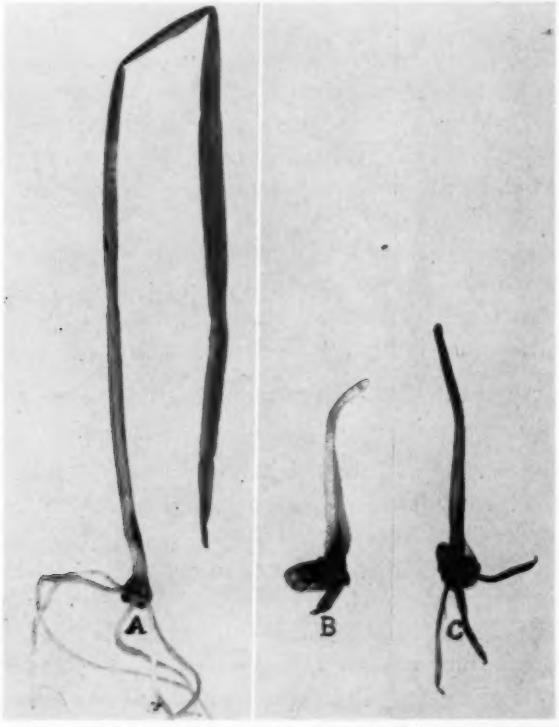

FIG. 1. Seedlings of Carleton durum inoculated with a single spore of race 1 of *Helminthosporium sativum* and grown on (A) sterile water agar, and (B, C) water agar with 1,000 and 3,000 p.p.m., respectively, of ammonium salt of 2,4-D.

Inoculated plants, after about 7 days on sterile agar, grew vigorously whereas those on water agar containing 1,000 p.p.m. of 2,4-D were stunted (Fig. 1). At higher concentrations of 2,4-D in the medium the inoculated plants were heavily infected. The fungus sporulated on the plants growing at all concentrations of 2,4-D. Noninoculated plants grown on agar containing similar amounts of 2,4-D were stunted by the chemical.

The predisposing effect of 2,4-D was tested in the greenhouse on Carleton durum. The inoculum of race 1 was increased on a grain medium, and a suspension of spores and mycelium was made from this medium after the fungus had been grown for about 2 weeks. Four-inch pots of autoclaved soil were inoculated with the suspension of spores and mycelium and planted with 25 seeds each. The control pots were moistened with water. Two concentrations, 1,000 and 5,000 p.p.m., of the ammonium salt of 2,4-D were prepared in distilled water. About 1 cc. of the solutions of 2,4-D was sprayed on each pot by means of an atomizer, either onto the soil immediately after planting or onto the plants 1 or 2 days after they had emerged from the soil. Control plots were sprayed with the same amount of distilled water. Each test was made in triplicate.

The plants grown on Helminthosporium-infested soil that was sprayed with 2,4-D immediately after planting were heavily infected and stunted (Fig. 2). The basal part of the stem was blackened from the infection, few roots developed, and the plants lacked vigor. Control plants were not so heavily infected, had well-developed roots, and were vigorous. Thus, increased infection on the inoculated plants treated with 2,4-D was evidently due to the predisposing effect of 2,4-D on the plants. Plants not inoculated with *Helminthosporium* but sprayed with 2,4-D also were definitely weakened. Applications of the chemical after the emergence of the plants had no appreciable effect on the plants nor on disease development.

TOLERANCE OF HELMINTHOSPORIUM SATIVUM TO 2,4-D

Both inhibitive and stimulative effects of 2,4-D on the growth of several fungi have been reported (1, 5, 7, 9). In present studies the tests were made on the effect of different formulations and concentrations of 2,4-D on growth of two races of *H. sativum*.

In a preliminary test, when the ammonium salt of 2,4-D was added to potato-dextrose agar, the growth of race 1 of *H. sativum* was inhibited in proportion to the concentration of this salt in the medium. Subsequently, two successive experiments were made with two races of *H. sativum* and four formulations of 2,4-D. The concentrations of the ammonium and the sodium salts of 2,4-D were 1,000, 5,000, and 10,000 p.p.m.; those of the amine and the ethyl ester were 100, 1,000, and 5,000 p.p.m. in one experiment and 100, 1,000, 5,000, and 10,000 p.p.m. in another.

A cork borer was used to cut uniform discs of mycelium from colonies of the fungus growing on potato-dextrose agar in Petri plates; thus uniform inocula were obtained from the two races of *H. sativum*. Triplicate plates for each concentration of each formulation of 2,4-D and for the controls of potato-dextrose agar without 2,4-D were inoculated. All cultures of each race were grown at room temperature, approximately 25° C.

Two effects of 2,4-D on the growth of *H. sativum* were noted. The ammonium and sodium salts of 2,4-D inhibited the growth of both races of *H. sativum* in proportion to the concentration of 2,4-D used. Race 1 was

FIG. 2. Effect of 2,4-D on the susceptibility of Carleton durum to race 1 of *Helminthosporium sativum* when grown on infested soil, (A) not sprayed and (B) sprayed with 5,000 p.p.m. of ammonium salt of 2,4-D immediately after planting.

more tolerant than race 2 to both ammonium and sodium salts of 2,4-D at 1,000 p.p.m. The amine and ethyl esters were both inhibitive and stimulative to the growth of race 1. The amine, at 100 and 1,000 p.p.m., stimu-

lated the growth of race 1 in the first experiment, where the average diameters of colonies of the triplicate plates were 80 and 84 mm. for 100 and 1,000 p.p.m. of the amine, respectively, that of the check being 73 mm. The ethyl ester, 100 p.p.m., also stimulated the growth. Both the amine and the ethyl ester at 5,000 and 10,000 p.p.m. decreased the growth of race 1. All concentrations of the amine and the ethyl ester inhibited the growth of race 2 in proportion to the concentration. Race 1 was more tolerant than race 2 to both the amine and the ethyl ester of 2,4-D at all concentrations.

Associated with the inhibition of the growth, there was a tendency toward the inhibition of sporulation proportional to the concentration of 2,4-D. However, a higher concentration of 2,4-D was needed to inhibit

TABLE 2.—*Total number of sectors produced in cultures by two races of Helminthosporium sativum, when grown on potato-dextrose agar containing different formulations and concentrations of 2,4-D*

Formulation of 2,4-D	Concentration (p.p.m.)	Number of variants[a]	
		Race 1	Race 2
None	0	0	0
Ammonium salt	1,000	1	0
	5,000	13	4
	10,000	3	3
Sodium salt	1,000	1	0
	5,000	5	3
	10,000	9	2
Amine	100	0	0
	1,000	0	0
	5,000	2	0
	10,000	1[b]	0[b]
Ethyl ester	100	0	0
	1,000	0	0
	5,000	0	0
	10,000	0[b]	1[b]

[a] Data are based on the average of duplicate tests on triplicate cultures for each formulation and each concentration of 2,4-D.

[b] Total number of sectors in one test.

sporulation than to inhibit growth. The ammonium and the sodium salts at 1,000 p.p.m., and the amine and the ethyl ester as high as 5,000 p.p.m., did not affect sporulation although they inhibited growth. Race 1 did not sporulate at 10,000 p.p.m. of the ammonium or sodium salt, whereas race 2 sporulated slightly.

EFFECT OF 2,4-D ON VARIATION IN HELMINTHOSPORIUM SATIVUM

In tests with the ammonium salt of 2,4-D on race 1 of *H. sativum*, 2,4-D not only inhibited the growth of the fungus but at certain concentrations it also induced sectoring, particularly on the medium with 5,000 p.p.m. of 2,4-D. Therefore, further investigations were made to determine the frequency of sectoring on different formulations of 2,4-D. The number of

variants in cultures of both races of *H. sativum* growing on potato-dextrose agar with different formulations and concentrations of 2,4-D is in table 2. The appearance of sectoring in cultures growing on two different formulations of 2,4-D is shown in figure 3.

Results indicate that sectoring was more common on the ammonium salt and the sodium salt than on the amine and the ethyl ester, occurring frequently on the former formulations but only occasionally on the latter. The concentration of 2,4-D, particularly of the ammonium salt and the sodium salt, in a medium also was important in the production of sectors. In race 1 sectoring on ammonium salt was more common at 5,000 p.p.m. than at 1,000 and 10,000 p.p.m., whereas that on the sodium salt occurred most frequently at 10,000 p.p.m. However, sectoring in race 2 on both ammonium and sodium salts occurred more frequently at 5,000 p.p.m. than at the other two concentrations,. The two races of *H. sativum* differed in their tendency to produce variants on a medium containing 2,4-D; variants were more common in race 1 than in race 2.

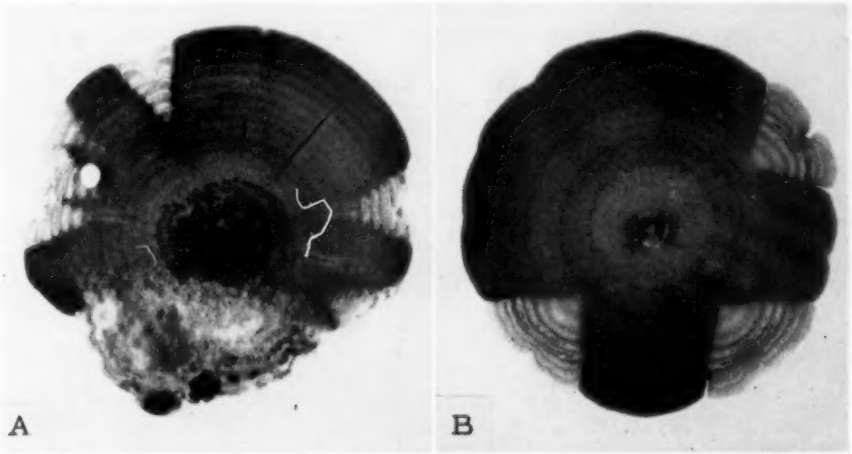

FIG. 3. Sectoring of race 1 of *Helminthosporium sativum* when grown on potato-dextrose agar containing 5,000 p.p.m. of (A) ammonium salt and (B) sodium salt of 2,4-D.

The variability of race 1 of *H. sativum* on a 2,4-D medium also was studied by the spraying method. A suspension of spores in sterile distilled water was made by taking spores from single-spore cultures growing on potato-dextrose agar with and without ammonium salt of 2,4-D (5,000 p.p.m.). The spores in suspension were then sprayed with an atomizer on potato-dextrose agar in Petri plates as suggested by Christensen and Schneider (4). Thirty-seven variant colonies appeared in the 660 colonies derived from the spores produced on the medium containing 2,4-D, whereas only one variant appeared in the 440 colonies obtained from the medium without 2,4-D.

In a similar manner the frequency of variation of *H. sativum* on the seedlings of wheat sprayed and not sprayed with ammonium salt of 2,4-D were ascertained. Sprayed and nonsprayed seedlings were inoculated in-

dividually with a single spore and then grown in test tubes containing water agar. Four of 4,988 colonies derived from spores produced on seedlings sprayed with 2,4-D differed strikingly from the others in cultural characters, whereas no variant occurred in 1,469 colonies derived from spores produced on the nontreated seedlings.

In general, 2,4-D inhibited the growth of *H. sativum* on a culture medium. Therefore it would be both important and interesting to know whether or not the variants had the ability to tolerate more 2,4-D than the parent. Race 2 of *H. sativum*, and its five variants which are morphologically different from the parent, were inoculated to potato-dextrose agar containing different concentrations of 2,4-D (Table 3).

Experimental results indicate that the variants differed from their parent and from each other in growth on 2,4-D medium, as indicated by the size of colony at certain concentrations. The most remarkable case is that

TABLE 3.—*Radial growth of race 1 of Helminthosporium sativum and its variants on potato-dextrose agar containing different concentrations of ammonium salt of 2,4-D*

Culture number	Diameter of colonies (in mm.)[a] at 2,4-D concentrations (p.p.m.):			
	None	1,000	5,000	10,000
Parent	75	56	24	20
1 Variant	75	53	36	39
2 do	48	59	36	19
3 do	44	71	57	49
4 do	70	65	33	30
5 do	78	54	35	26

[a] Data are based on averages of triplicate cultures.

both the variant 1 and the parent produced colonies of the same size on potato-dextrose agar, but the colonies of variant 1 at 10,000 p.p.m. were approximately twice as large as those of the parent at the same concentration. Variant 3 also had greater colony size than had the parent at all concentrations of 2,4-D, although its diameters of colonies on potato-dextrose agar were much less than those of the parent on the same medium.

The pathogenicity of the variants of race 1 of *H. sativum* on Mida wheat was tested in the greenhouse. The methods used in inoculation and in planting were the same as given under the experiment for investigating the pathogenicity of *H. sativum* grown on a 2,4-D medium. There was a gradation in the pathogenicity of different variants, ranging from almost avirulence in variant 2, to high virulence in variant 1 (Table 4).

DISCUSSION AND CONCLUSION

The present investigation indicates that 2,4-D increased the susceptibility of wheat plants to *H. sativum*. It is obvious from the evidence obtained that the increase in susceptibility was due to the predisposing effect of 2,4-D on the host plant rather than to an increase in the virulence of the

pathogen. When the fungus was grown on a medium containing 2,4-D, and the resultant inoculum was washed with water to remove the 2,4-D, the infection of the plants was less than when the inoculum was increased on the same medium but not washed. Second, the greenhouse test indicates that wheat plants treated with a certain amount of 2,4-D on the soil immediately after planting were definitely weakened, as judged by general vigor and the green weight of the plants. When plants were inoculated with *H. sativum*, infection on the plants grown on soil treated with 2,4-D was heavier than that on the checks. Third, in laboratory tests seedlings grown on water agar containing 2,4-D became stunted, distorted, and hypertrophic in certain areas, and were infected by *Helminthosporium* earlier and more heavily than those grown on water agar lacking 2,4-D.

Previous workers have shown that the variation of *H. sativum* in culture can readily be induced by means of physical and chemical treatments. Evidence in the present work indicates that genetic variation of this fungus

TABLE 4.—*Pathogenicity of Helminthosporium sativum race 1 and its variants, induced by 2,4-D on Mida wheat*

Culture number	Percentage of plants emerged[a]	Degree of root infection	Green weight (gm.)
Check	92	None	8.3
Parent	8	Heavy	0.8
1 Variant	12	do	0.5
2 do	88	Trace	7.3
3 do	28	Heavy	0.8
4 do	48	Slight	4.0
5 do	24	Heavy	1.0

[a] The results are based on triplicate pots.

occurred on a cultural medium containing 2,4-D and that some concentrations and formulations of 2,4-D were more effective than others in the production of sectors. Certain sector-variants tended to be more tolerant to 2,4-D at certain concentrations than was the parent. None of the five variants tested for pathogenicity was more pathogenic than the parent.

Applications of 2,4-D as an herbicide in the field have been very common in recent years. The evidence obtained in the present investigation led to the conclusion that the use of 2,4-D as a weed killer in the field may aid under certain conditions in the development of diseases caused by *Helminthosporium* and perhaps in the production of new races of the pathogen.

SUMMARY

The pathogenicity of *H. sativum* on Mida wheat was increased by growing the inoculum on 2,4-D medium, and evidence indicates that the results were due to the predisposing effect of 2,4-D on host plants rather than to an increase in the virulence of the pathogen.

Wheat plants treated with 2,4-D were definitely weakened, hypertro-

phied, and stunted and, when inoculated with *H. sativum*, were more heavily infected than those not treated.

Growth of *H. sativum* on a culture medium was in most cases inhibited by 2,4-D; in a few cases it was stimulated. The inhibitive effect of 2,4-D increased with an increase in the concentration.

Genetic variation occurred in *H. sativum* on a 2,4-D medium, and appeared as sectors in the colony. Frequency of sectoring differed with the formulations of 2,4-D, concentrations of 2,4-D, and races of the fungus. Certain variants were found to be more tolerant than their parent to 2,4-D at certain concentrations; none was more pathogenic than the parent.

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NOTES ON THE PHYSIOLOGY AND PATHOGENICITY OF CENTROSPORA ACERINA (HARTIG) NEWHALL

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(Accepted for publication August 15, 1951)

The intercontinental occurrence of this fungus, its wide host range, its persistence in organic soils, and its damage to both ornamental and vegetable crops warrant a summary at this time of what is known of its physiology and parasitism. Part of the data recorded here for the first time was obtained in 1946 by both authors working together at Cornell, and part is the result of work done in Denmark and in New York State by each independently.

DISTRIBUTION AND ECONOMIC SIGNIFICANCE OF THE PATHOGEN

The fungus by one name or another has been recorded from Germany by Hartig (3), France by Arnaud (2), Switzerland by Osterwalder (10), Holland by Westerdijk and van Luijk (17), Denmark by Neergaard (5), Canada by Truscott (15), the United States and Alaska by Newhall (8) and Tompkins and Hansen (14), and Norway by Jørstad (4).

Hartig pointed out that heavy losses of maple seedlings were sustained in the spring in Bavarian nurseries from attacks of this fungus, which he called *Cercospora acerina*. The junior author has seen 50 per cent of some lots of cold-storage celery in western New York State rendered worthless by the end of December in some seasons, which meant the loss of many thousands of dollars. Truscott (15) reports losses to certain lots of stored celery in Canada up to 100 per cent by late February. Tompkins and Hansen (14) reported over a million pansy plants lost annually by growers in two counties in California by 1940. Neergaard has estimated losses from root rot on *Primula malacoides* in one Danish nursery in Copenhagen in the five seasons from 1944 to 1948 from 12,000 to 25,000 Danish kroner, representing more than 50 per cent of the entire crop in the latter case. Westerdijk and van Luijk (17) reported the fungus killing caraway plants in Holland in 1924, but gave no estimate of the loss sustained.

NOMENCLATURE

Since a comprehensive review of the names applied to this fungus has already appeared in this publication (9), it is necessary only to add a tenth combination to the list: *Centrospora macrospora* (Osterw.) Neerg., published by the senior author (6) in 1943 and overlooked in the review just mentioned. In brief, the fungus was first named *Cercospora acerina* by Hartig (3) in 1880 and was given generic rank and renamed *Centrospora*

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ohlsenii by Neergaard (5) in 1942, the proper recombination of *Centrospora acerina* (Hartig) being proposed by both Newhall (9) and Viennot-Bourgin (16) independently.[3] Unfortunately Newhall (8) had also raised it to generic rank, calling it *Ansatospora macrospora* (Osterw.). The complete list of synonyms to date now includes:

Cercospora acerina Hartig.
Sporidesmium acerina (Hart.) Frank
Cercosporella acerina (Hart.) Arnaud
Cercospora macrospora Osterwalder
Cercospora cari Westerdijk & v. Luijk
Cercospora praegrandis Sprague
Centrospora ohlsenii Neergaard
Centrospora macrospora (Osterw.) Neergaard
Ansatospora macrospora (Osterw.) Newhall
Ansatospora acerina (Hart.) Hansen & Tompkins

MORPHOLOGY

The morphological characters of this fungus have been thoroughly described and illustrated by Westerdijk and van Luijk (17), by Neergaard (5), and by Newhall (8). A good illustration of the conidia is given by Tompkins and Hansen (14).

The principal characters by which *Centrospora* is distinguishable include its rapidly growing mycelium of variable size, 6 to 9 μ, always becoming very torulose, dark, and broad (up to 30 μ) with age, with large obclavate multiseptate hyaline conidia, 105–210 × 6–11 μ, similar to those of *Cercospora* but larger, much broader, and gradually narrowing, whiplike, toward the apex and usually with a swordlike appendage 2 × 90 μ hanging down from the basal cell. This latter feature is not present on all spores, at least not all of the younger ones. The probable importance to the fungus of the thick-walled chlamydosporelike cells of the older mycelium as the overwintering stage has been indicated by experiments to be described. Truscott states that these cells are "viable after lengthy periods of desiccation." Under proper moisture and temperature conditions conidia can arise from such fragments.

PHYSIOLOGY

Spore production. The fungus is easily isolated from bits of infected host tissue on a variety of agar media. On potato-dextrose agar and cornmeal it grows rapidly, but several workers have had difficulty in obtaining many spores. Tompkins and Hansen used pea-straw agar and incubated their cultures outside a north window at Berkeley, California, but this method was not successful in Denmark. Westerdijk and van Luijk suggested turning over the agar in a well-grown Petri-dish agar culture, letting the fungus grow through to the surface of the medium where conidia are

[3] In 1945 according to his paper, which was published, however, December 1, 1946.

borne. This is more or less successful with fresh isolates, but old ones seem to lose their capacity to sporulate.

The writers achieved some success at inducing sporulation by cutting out pieces of agar cultures and placing them on the underside of the cover of a Stender dish with 1 in. of water in the bottom. Under these conditions conidia were formed within 3 days at 18° C. by cultures derived from parsley, celery, carrot, and muck soil. At another time in place of water the dishes held sulfuric acid solutions of the proper concentrations to maintain humidities of 97, 94, 90, 86, 84, 82, and 80 per cent respectively. On the three isolates employed, from parsley, celery, and carrot, conidia were formed around the cut edge of the agar after 48 hours' incubation at 15° C., at all the above humidities, more abundantly at the highest.

The fungus was transferred to several Petri dishes of Difco cornmeal agar made up at concentrations of 0.75, 1.5, and 3 per cent agar. Two plates of each concentration were incubated for 6 days, December 6 to 12, at each of four temperatures: 12°, 15°, 18°, and 21° C. No spores were found, and the agar in one of each pair was cut vertically and also scraped in several places. Later examination revealed no sporulation in any of the dishes regardless of treatment. The reason for the failure to obtain spores has remained undisclosed but it is not density of the agar.

Scraping the surface of the cornmeal agar cultures in Petri dishes and cutting out narrow slivers of the agar induced sporulation on fresh isolates, but not after a few months in culture.

One of the best ways of procuring spores for inoculation was that of placing small pieces of infected plant tissue (roots of *Primula malacoides* or bits of celery stalk) in drops of sterile water on slides laid in Petri dishes. After incubation at room temperature for a day or two, conidia formed abundantly on the surfaces of the drops.

Failure of several efforts to obtain pure cultures by inverting bits of sporulating host tissue over poured agar plates indicates that the conidia are not forcibly and perhaps not even easily detached from their conidiophores.

Longevity. The longevity of conidia was studied in the laboratory at room temperatures by placing drops of spore suspensions on sterile cover slips and inverting these, after various periods of drying, on potato-dextrose agar. As long as there was a slight film of water still on the cover, growth could be secured, but none was ever obtained from dried spores even though the cover slip was inverted only a few minutes after drying was complete.

Relation of temperature and pH to growth in culture. Centrospora acerina might be expected to have a low optimum temperature for growth when we consider how devastating can be its effect on celery and on carrots held at 0° C. in cold storage. The cardinal points are indicated by the graph in figure 1. No growth occurred at 30° C., optimum appeared to be close to 17°, and enough growth occurred at the lower range to verify

cold-storage observations indicating its ability to grow at subfreezing temperatures.

From comparative growth rates of *Centrospora acerina* and *Cercospora apii* on potato-dextrose agar adjusted to various hydrogen-ion concentrations (Fig. 2), it can be seen that not only does *Centrospora* grow more

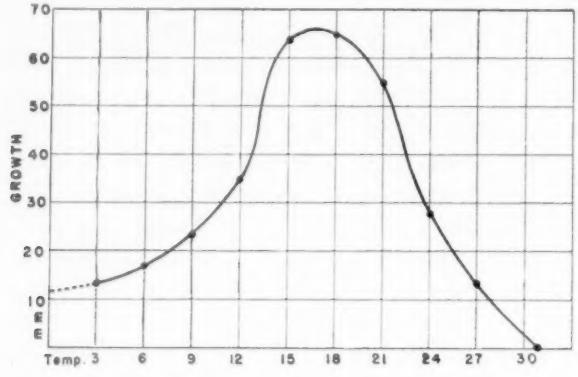

FIG. 1. Temperature growth curve for *Centrospora acerina*, 9 days on 2 per cent potato-dextrose agar.

than twice as fast but that it has a higher, narrower optimum range than does *Cercospora*.

Another celery pathogen occurring in Europe and America and which somewhat resembles *Centrospora* vegetatively is *Phoma apiicola*. But as the graphs for growth rates in figure 3 show, *Phoma* is a very much slower growing fungus at 18° C.

Pigment production. Some cultures develop a red pigment; others

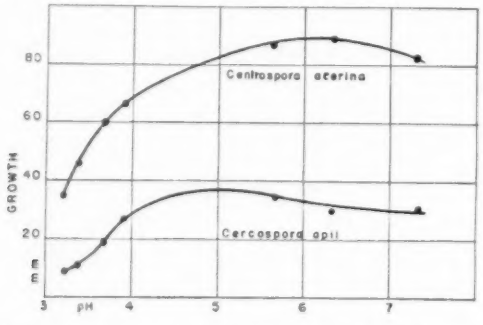

FIG. 2. The pH-growth curves of *Centrospora acerina* and *Cercospora apii*, 10 days at 24° C. on potato-dextrose agar.

form concentric rings of green and red as daily growth occurs. Still others will be solid dark green, and some of these may have red sectors in them. Truscott states that red is more apt to be the color if cultures are grown above 80° F., and green if held at 65°. He also says cultures receiving 4–15 drops of a 14 per cent solution of borax were pink.

More precisely designated, the color is a Rhodonate red (0022/2–3) or

Spinel red (0023/2–3) in the Horticultural Colour Chart, London (1, pp. 188–189), or according to Ridgway (12), a Eugenia red, plate XIII, or Spinel red, plate XXVI. The interesting thing is that this pigment production is a response to light, as demonstrated in Denmark by the experiment described below.

Fresh transfers June 5 of the type and the isolate I to malt-extract agar in Petri dishes were immediately wrapped in opaque paper to exclude light, except for one unwrapped control. After 4 days' incubation the dishes were unwrapped and exposed to daylight for 4, 8, 16, 32, 64, 128, 256, and 512 min., respectively. One was kept wrapped for another control. The first trace of pigment appeared in the first unwrapped control 48 hr. after the Petri dish was inoculated. On June 11, at 3 p.m., 2 days after the exposures to daylight were made, all were examined under an electric light during a 3-min. period, and a circle of red pigment was observed on all dishes previously exposed to daylight. This colored band was faint and

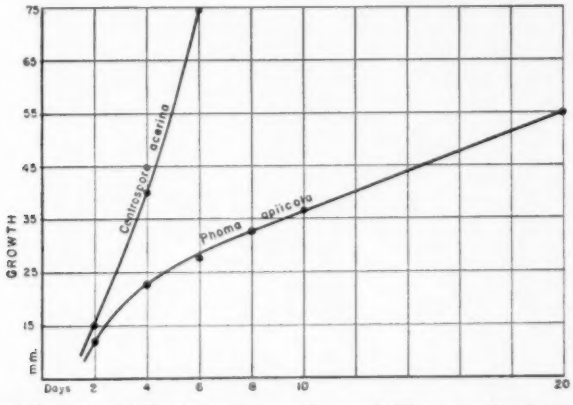

FIG. 3. Growth rates of *Centrospora acerina* and *Phoma apiicola* on cornmeal agar at 18° C.

narrow on the dish exposed 4 min. and correspondingly strong and broad on the one exposed 8½ hr. Repeated exposures to daylight produced a series of concentric rings of pigment (Fig. 4). The color never appeared until several hours after the exposure to daylight and was localized in the part of the medium in which the fungus made growth during the exposure period. Diffusion of the pigment into the uncolored interzones took place in a few days. Wrapped and unwrapped controls remained, respectively, colorless and uniformly red after the end of the experimental period.

It was also observed that when certain isolates or strains of the fungus were placed in test tubes in which seedlings were growing on filter paper, intense red to Mars orange pigment was produced in the presence of *Brassica oleracea* or *Primula malacoides*, a fainter color in the presence of *Lactuca sativa*, and none in the presence of *Lycopersicon esculentum* (Table 1). There seemed to be no connection between susceptibility or virulence and

pigment production in these experiments, which is in agreement with the observations of Truscott.

The chemistry or mechanism of this pigment production is still obscure.

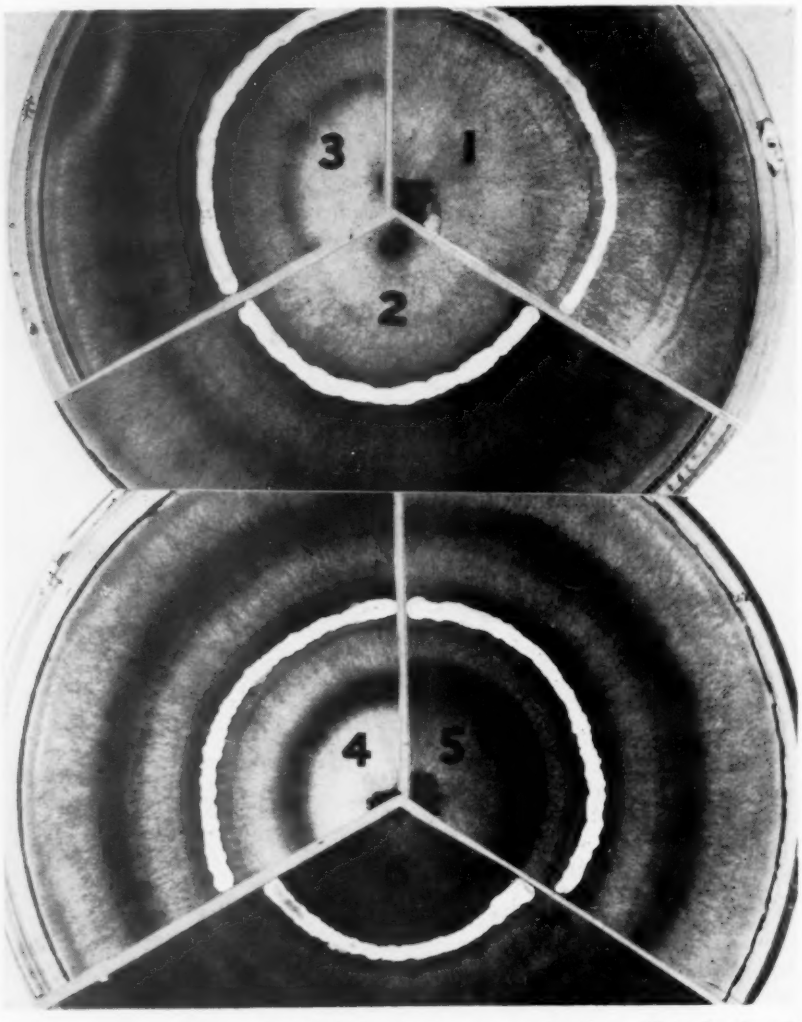

FIG. 4. Demonstration of photoinduced pigment production. Note bands of pigment within white circles. The narrow fine line close to the white circle is the result of 3-min. exposure to electric light June 11. The wide black band within this, easily seen in sections 4 and 5, is the result of daylight exposures June 9. 1, unexposed check; 2, exposed for 4 min.; 3, for 64 min.; 4, for 256 min.; and 5, for 512 min.; 6, continuous light.

It obviously depends upon light. Even a 3-min. exposure to electric light causes a reaction observable in several of the plates in figure 4 as a band 0.5 mm. wide. But it remains to be determined whether the pigment is produced directly from the hyphae of the fungus or whether the hyphae under

certain circumstances (depending on isolate, host, etc.) produce a colorless substance from which the pigment is developed by photoinduction.

Crystal production. Isolate I and Isolate II, when grown on malt extract agar, produced large crystal aggregates measuring 2–4 mm. long and consisting of numerous needle-shaped crystals.

PATHOGENICITY

Infection experiments. Infection experiments with sterile seedlings grown in test tubes as described by Neergaard (7) were performed by the senior author in 1948 and 1949. These show that the fungus is an extremely polyphagous parasite numbering among its hosts such unrelated plants as *Allium cepa, Brassica oleracea, Daucus carota, Dianthus caryophyllus, Godetia hybrida, Lactuca sativa, Lycopersicon esculentum,* and *Primula malacoides.* Tables 2 and 3 give the numbers of seedlings attacked and degrees of infection secured in experiments (see 7, p. 49).

TABLE 1.—*The influence of four hosts on pigment production of three isolates of Centrospora acerina from Primula (24 March 1949)*

Host	Isolate[a]	Numbers of test tubes showing color			Color index[b]
		None	Faint	Strong	
Brassica oleracea	I	4	4	2	8
	II	1	7	2	11
	III	10	0	0	0
Lycopersicon esculentum	I	9	1	0	1
	II	10	0	0	0
	III	10	0	0	0
Lactuca sativa	I	6	4	0	4
	II	8	2	0	2
	III	10	0	0	0
Primula malacoides	I	3	7	0	7
	II	1	3	6	15
	III	10	0	0	0

[a] I, a culture from *Primula malacoides* isolated March 11, 1948; II, a culture from *P. malacoides* isolated February 14, 1949; III, a culture from *P. veris* isolated December 20, 1948.

[b] Index = Number none × 0 + number faint × 1 + number strong × 2. Max. possible, 20.

In a similar experiment comparing the pathogenicity of three isolates on four hosts, the results in table 3 were obtained.

From a comparison of the data in tables 2 and 3, isolate I, from *Primula,* lost a good deal of its virulence from November 1948 to March 1949, since it failed to attack any of the four hosts employed in the second experiment to the extent that it did in the first one. Further evidence of diminished virulence with time is afforded by a comparison of the behavior of isolate I 8 months after its isolation from *P. malacoides* and a culture isolated 6 years previously from *Petroselinum crispum* var. *radicinum.* Of 27 seedlings of *P. malacoides* inoculated with isolate I, 20 were either killed or

severely attacked (Table 2), whereas of 28 inoculated with the older isolate, only six were killed or severely attacked. Truscott (15), however, states

TABLE 2.—*Infection results with Centrospora acerina "I," isolated March 11, 1948, from Primula malacoides, on seedlings of nine host plants 12 days after inoculation November 10, 1948*

Plants inoculated	Degrees of infection and numbers of seedlings in each class						
	Checks		Inoculated				
	Healthy	Total	Dead	Severe	Light	None	Total
Daucus carota	35	35	3	3	2	28	36
Brassica oleracea	43	43	4	9	16	12	41
Dianthus caryophyllus	45	45	1	2	5	26	34
Pisum sativum	43	43	0	2	2	40	44
Allium cepa	42	42	4	3	4	34	45
Lycopersicon esculentum	41	41	5	12	9	8	34
Lactuca sativa	42	42	9	11	6	14	40
Godetia hybrida	10	10	0	2	3	3	8
Primula malacoides	29	29	12	8	3	4	27

that his strain from celery lost none of its pathogenicity for that host in 4 years if protected from desiccation.

HOSTS

The following is a list of hosts reported as susceptible. An asterisk indicates those found naturally infected in the field; others were experi-

TABLE 3.—*Infection results with three Centrospora isolates on four hosts 12 days after inoculation, March 24, 1949*

Isolate, and plants inoculated[a]	Degrees of infection and numbers of seedlings in each class				
	Dead	Severe	Light	None	Total
I (from *Primula malacoides*, 1948)					
Brassica oleracea	0	1	13	34	48
Lycopersicon esculentum	0	0	1	43	44
Lactuca sativa	0	0	0	37	37
Primula malacoides	7	1	3	16	27
II (from *Primula malacoides*, 1949)					
Brassica oleracea	4	15	25	4	48
Lycopersicon esculentum	0	4	18	18	40
Lactuca sativa	1	1	2	35	39
Primula malacoides	24	1	4	1	30
III (from *Primula veris*, 1948)					
Brassica oleracea	0	3	10	36	49
Lycopersicon esculentum	0	0	0	40	40
Lactuca sativa	0	0	0	41	41
Primula malacoides	4	0	2	18	24

[a] None of the 157 uninoculated checks became infected.

mentally infected in the greenhouse or laboratory. Numbers in parentheses are literature references; an N in parentheses indicates that Neergaard is

reporting the host for the first time from work in Denmark.[4] Tompkins and Hansen (14) also list 22 ornamental bedding plants, the inoculation of which was unsuccessful.

**Acer pseudoplatanus*, Norway maple (3, 8)
Allium cepa, onion (N)
**Apium graveolens*, celery (8, 14, 15)
**Aquilegia* sp., columbine (14)
Begonia semperflorens, begonia, fibrous (14)
Beta vulgaris, beet (11, 14, 15)
Brassica chinensis, Chinese cabbage (14)
Brassica napobrassica, rutabaga (15)
Brassica oleracea, cauliflower (14)
Brassica oleracea, cabbage (14) (N)
Callistephus chinensis, China aster (14)
**Campanula medium*, Canterbury bell (14)
**Carum carvi*, caraway (17)
**Centaurea cyanus*, cornflower (14)
Chrysanthemum coccineum, painted daisy (14)
Chrysanthemum maximum, Shasta daisy (14)
**Cichorium intybus*, French endive (14)
Cyclamen indicum, cyclamen (14)
Daucus carota, carrot (11, 14, 15) (N)
**Delphinium cultorum*, delphinium (14)
Dianthus caryophyllus, carnation (N)
Geranium sanguineum, geranium (14)
Gerbera jamesonii, Transvaal daisy (14)
Geum chiloense, geum (14)
Godetia hybrida, godetia (N)
Helipteron roseum, acroclinium (14)
**Iris germanica*, iris (4)
Lactuca sativa var. *capitata*, lettuce (14) (N)
**Lactuca sativa* var. *romana*, lettuce (14)
**Lobelia hybrida*, lobelia (14)
Lycopersicon esculentum, tomato (14) (N)
Malcomia maritima, Virginia stock (14)
Myosotis alpestris, forget-me-not (14)
**Omphalodes linifolia*, Chinese forget-me-not (14)
Osmorhiza brevipes, osmorhiza (13)
**Pastinaca sativa*, parsnip (14)
**Penstemon barbatus*, penstemon (14)
**Petroselinum crispum* var. *radicinum*, parsley (rooted) (5)
Petroselinum crispum (as *P. hortense*), parsley (14)
**Petunia hybrida*, petunia (14)
Phaseolus vulgaris, bean (14)
Pisum sativum, *pea* (14) (N)
**Primula malacoides*, primula (N)
Primula obconica, primula (14)
**Primula veris*, primula (N)
**Ranunculus asiaticus*, ranunculus (14)
Rhododendron indicum, azalea (14)
**Scabiosa atropurpurea*, scabiosa (14)
Senecio cruentus, cineraria (14)
Solanum tuberosum, potato (15)
**Taraxacum officinale*, narrow-leaf dandelion (14)
Vicia faba, horsebean (14)
**Viola cornuta*, horned violet (4, 14)
**Viola tricolor*, pansy (10, 6, 14)

LIFE CYCLE

Perfect stage not found. Although the fungus has been grown on various liquid and solid media including sterilized plant materials, and on

[4] As this paper was going to press, word came of an article entitled "Centrospora root rot, a serious disease of Primula malacoides in Denmark," by Paul Neergaard, to appear in Acta Agriculturae Scandinavica 1: 1951.

sterile plants of various species, and although it has been irradiated, and grown in the dark and at various temperatures and humidities and pH levels, a perfect stage has not been found.

Overwintering, recovery of Centrospora from muckland soil. Truscott (15), Newhall (8), and Rader (11) all concluded that soil must be the source of primary inoculum in the initial infections in the case of celery black crown rot, and of licorice storage rot of carrot. Truscott, however, was unsuccessful in attempts to isolate *Centrospora* from Thedford muck soils. Feeling sure that the fungus must be present in certain muckland fields in Wayne County, New York, where celery black crown rot was a frequent problem, the writers employed a simple technique which clearly proved the presence of viable bits of mycelium in soil samples from two of eight fields sampled.

On October 9 samples were collected from the upper 6 in. of soil in several muckland fields. Five days later, 2 per cent acidified potato-dextrose agar was poured into several Petri dishes and ½ teaspoonful of suspected muck was dusted over the surface of each as the agar solidified. After 7 days' incubation at 15° C., the dishes were inverted and examined through the bottoms with a low-power binocular microscope. From one to five submerged colonies of *Centrospora* were found in five of six dishes representing one of the Wayne County fields, being easily distinguished by the large, dark, torose mycelium growing through the agar. When a small bit of this mycelium was carefully removed with a sterile needle and placed on an acidified-agar slant, the fungus was recovered. Bits of the resulting agar culture were inverted over water in a Stender dish; spore formation was observed a few days later and the fungus was identified as *Centrospora*.

This method of recovery directly from the soil was repeated successfully with another sample collected October 17 from a celery muck farm in Cattaraugus County, New York. However, the fungus was not recovered from six other field samples collected October 9 but permitted to become very dry in the ensuing 2 months before the tests were made. In this attempt one of the samples was an aliquot portion of the Wayne County sample previously shown to be infested, which leads to the conclusion that even the thick-walled torose mycelium cannot stand very much desiccation. This conclusion is further supported by the following experiences: 1) cultures are lost when not transferred frequently enough, 2) quickly dried agar cultures lose their viability in less than 40 days, and 3) the fungus cannot stand lyophilization even for a few days.

Further proof that the soil harbors the primary inoculum was afforded by an experiment in which the butt ends of freshly cut but disinfected celery plants were inoculated with a dab of wet muck on November 8, a month after collection of the soil samples. Eight soil samples were tested on five plants each, one sample being from the same field in Wayne County from which the fungus was successfully isolated by the agar plate method. A boiled sample of muck was used on eight plants, an agar culture on six,

and five were untreated checks making 59 total or enough for one celery crate. The crate was covered with a cellophane wrapping to hold in moisture and was placed in cold storage at 32° F. On January 13, 1947, after 2 months' storage, positive infections were observed from inoculations made with the muck soil sample from Wayne County and with pure cultures derived from celery and from carrot. In 2 months' time the lesions had progressed from 1 to 3 in. up the stalks of the five infected plants. None of the checks became infected. It seems quite certain, therefore, that primary infections can take place as a result of soil-borne inoculum, and it is likely that the fungus was not present or was no longer viable in the other seven soil samples used in the above experiments, perhaps because the soil had dried too much in the month since it had been collected.

It is probable that conidia arise from the resting or overwintering mycelium in the soil, though how important they are in view of their delicate nature and short life when dried may be questioned. But it is of interest that after an agar culture was homogenized with distilled water in an electric fluid blender for 1 min. and stored 3 days at room temperature, young hyaline mycelial branches grew out from many of the dark, old, torose mycelial fragments. When small portions of this suspension were held in shell vials for nearly a month, a good many typical conidia were found floating on the liquid. It would seem highly possible for similar sporulation to occur during prolonged moist conditions out-of-doors in or on infested soil.

Seed transmission. Although no one has proven that *Centrospora* is seed-borne, one might postulate that it is, from the following facts. It has been seen growing and fruiting on the cotyledons of *Acer pseudoplatanus.* Westerdijk (17) found it attacking stems and leaves of caraway seedlings grown in the fall from seed sent to her by a farmer who had lost heavily from the disease. Neergaard (6) found the fungus on immature seeds in capsules of pansy. Truscott has pointed out that the fungus seems to have a preference for mature tissue, which at least would not rule out the possibility of seed-coat infection. Because the lack of fruiting bodies in culture would make identification difficult by seed laboratory techniques in common use, the fungus may have been overlooked.

CONTROL MEASURES

Newhall (8) tested a dozen fungicidal dips for control of celery crown rot at harvest time and found evidence of control of storage rot only with a solution of ethyl mercury chloride at 1 part in 400 of water. Copper sulphate 1:30 seemed to increase the amount of subsequent rot. In similar trials with 48 chemical dips, Truscott (15) also found a mercurial the most effective. Phenyl mercuric acetate was toxic *in vitro* at 1:8000 and as a dip for freshly cut celery at 1:4000. The junior author, in 1946, employed this chemical at 1:4000 in one farm experiment on 50 crates of celery as they were brought from the field, unwashed, ready for cold storage. The

shallow pans of fungicidal solution in which the crates were dipped to a depth of 2 in. soon became fouled with muck soil adhering to the celery, and the failure of the solution to give an appreciable measure of control is believed due to the rapid reduction in toxicity of the solution, which Truscott showed could take place in the presence of soil. Employment of this technique, therefore, can be successful only if celery is washed before dipping and storing. The process of washing is usually postponed in New York State until the end of the cold-storage period, so that dipping in a mercurial, in addition to the health risk involved, is not very practicable. The marketing of stored celery grown on infested soil, as soon as the first symptoms of butt-end decay show up, is considered the most recommendable practice for celery growers at present.

The leaf-spot disease of pansy and viola, according to Tompkins and Hansen (14), has been successfully controlled in the San Francisco Bay area by spraying the growing plants during the rainy season, after or between rains, with a dilute copper oleate fungicide.[5]

SUMMARY

Centrospora acerina now occurs in most European and North American countries.

Spore production was induced when pieces of infected plant tissue were inverted over water or sulfuric acid solutions in closed containers, in humidities as low as 80 per cent.

Conidia, on the other hand, were unable to retain their viability on glass slides after a few minutes of drying in laboratory air.

Centrospora acerina from celery grew much faster than either *Phoma apiicola* or *Cercospora apii* from the same host, and had a higher, narrower optimum pH range on potato-dextrose agar than the latter.

Exposure to natural or electric light induces the formation of a Spinel red pigment by *Centrospora* on malt extract agar. Pigment production was influenced by different host plants, being a red to Mars orange in the presence of seedlings of *Primula malacoides,* a fainter color in the presence of *Lactuca sativa,* and absent in the presence of *Lycopersicon esculentum.* Large crystal aggregates are produced in the agar.

Infection experiments with Centrospora show it to be an extremely polyphagous parasite with host plants as unrelated as *Allium cepa, Brassica oleracea, Daucus carota, Dianthus caryophyllus, Godetia hybrida, Lactuca sativa, Lycopersicon esculentum,* and *Primula malacoides.*

All attempts to find a perfect stage have failed.

The fungus was successfully isolated directly from muckland soil for the first time when ½ teaspoonful of suspected soil was dusted over the surface of potato-dextrose agar and the agar was searched, 7 days later, with low-power binocular microscope, for colonies with characteristic dark, broad,

[5] The trade names of two such fungicides are said to be Greenol and Mil-Du-Spra, made by California Spray Chemical Company and Shell Oil Company, respectively.

torose mycelium deep in the agar. Removal of a bit of such mycelium, culturing, and inducing spore production completed the recovery.

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PHYTOPATHOLOGICAL NOTES

Antibiotic Potentialities of Some Strains of Streptomyces scabies. PETER A. ARK AND J. W. OSWALD. It is well known that many species of *Streptomyces* yield valuable antibiotics such as streptomycin and aureomycin. However, as far as the authors are aware, there has been no report on any antibiotic activity in *Streptomyces scabies.*

Working with some 40 strains of *S. scabies* the authors observed strong antibiotic tendencies among several of them. The activity of different strains was tested on *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) and on the fungi, *Verticillium albo-atrum* and *Fusarium oxysporum.* The scab organisms were grown for 2 days on potato-dextrose agar plates, after which agar discs were made with a cork borer

TABLE 1.—*Zones of inhibition by Streptomyces scabies*

Strain	Pathogenicity	*S. aureus*	*E. coli*	*V. albo-atrum*	*F. oxysporum*
368-J	None	6	0	7	7
374-1B	Severe	12	0	10	12
386-2B	Severe	8	0	6	0
396-1B	Weak	10	0	12	14
417-A	None	20	0	18	20
417-B	None	15	0	13	14
417-C	None	6	0	0	0
417-D	None	15	0	12	10
419	Weak	20	0	20	19

3 mm. in diameter from the area closely adjacent to the colony of the organism. The bacterial test organisms were grown in poured plates of beef-extract-peptone agar at 37° C. and the fungi in potato-dextrose agar at 28° C. The discs were placed on the surface of agar-poured plates seeded with the test organisms.

The zones of inhibition produced by the strains of *S. scabies* (Table 1) were measured within 24 hr. for the bacteria and 4 days for fungi, and expressed in millimeters.

Some strains of *Streptomyces scabies* exerted strong antibiotic action against the Gram positive *S. aureus* and the fungi.

It is interesting to note that most of the strains of *Streptomyces scabies* used in testing and which showed strong antibiotic activity were cultural mutants from strains that originally were very pathogenic.—Division of Plant Pathology, University of California, Berkeley and Davis, California.

Varietal Reaction to Dwarf Bunt and Flag Smut, and the Occurrence of Both in the Same Wheat Plant.[1] C. S. HOLTON AND THOMAS L. JACKSON.[2]

Dwarf bunt (*Tilletia caries* (DC.) Tul.) and flag smut (*Urocystis tritici* Köern.) have occurred simultaneously in fields of winter wheat in Klickitat County, Washington, since 1940. Surveys beginning that year show that flag smut has occurred in about one-half the fields in the infested area covering 100 square miles, the incidence ranging from a trace to 10 per cent of infected plants. In occasional fields, spots of an acre in size had as high as 25 per cent of smutted plants. Dwarf bunt, although equally persistent during this period, was less severe until 1950.

In 1950, severe infestations of both dwarf bunt and flag smut occurred in at least two fields of wheat near Goldendale, Washington. In one of these fields, seeded to Hybrid 143 (C.I. No. 4160), 20 per cent of the heads had dwarf bunt and 15 per cent of the plants had flag smut. Fortunately a yield nursery of winter wheat varieties had been sown in this field by the junior author, and conditions favored the development of both smuts. The varietal reaction to dwarf bunt and flag smut and the occurrence of both smuts in the same plant are reported here.

TABLE 1.—*Dwarf bunt and flag smut in 14 varieties and strains of winter wheat at Goldendale, Washington, in 1950*

Variety or strain	C. I. Number	Percentage of heads infected with dwarf bunt	Percentage of plants infected with flag smut
Golden	1063	15	0
Rio	10061	48	Tr
Orfed	11913	15	2
Hymar	11605	0	9
Rex	11689	Tr	Tr
Elgin	11755	26	4
Elmar	12392	Tr	4
Uma	12247	5	1
Orfed × Hybrid 1-3	12688	0	3
Orfed × Hybrid 1-6	12689	1	3
Orfed × Elgin -1	.....	42	4
Orfed × Elgin -3	.....	49	5
Oro × (T-F) Elgin -19	12393	22	13
Oro × (T-F) Elgin -30	12394	11	6

The winter wheat yield nursery consisted of 14 varieties sown in randomized rod-rows replicated five times. The percentages of dwarf bunt

[1] Cooperative investigations of the Washington Agricultural Experiment Stations and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. Published as Scientific Paper No. 1008, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

[2] Pathologist and assistant agronomist, respectively, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dept. of Agriculture, and Washington Agricultural Experiment Stations.

and flag smut were computed from counts of infected heads and plants, respectively, in all replications. The data are presented in table 1. Varieties with less than 5 per cent dwarf bunt or less than 1 per cent flag smut are classed arbitrarily as resistant. This classification is based on the usual occurrence of the two smuts in varieties of known resistance or susceptibility.

Rex was the only variety resistant to both smuts (Table 1). Four varieties were resistant only to dwarf bunt, two were resistant to flag smut, and the remainder were susceptible to both. The resistance of Rex to flag smut in the field is remarkable, since it is highly susceptible in the greenhouse. Rex had 52 per cent smut in one greenhouse test and 63 per cent in another. No more than a trace of flag smut has been observed in commercial fields of Rex.

FIG. 1. Two Orfed × Elgin -1 plants infected with both flag smut and dwarf bunt.

Dwarf bunt and flag smut can best be controlled by growing resistant varieties, because both smuts develop from soil infestation. The need for varieties with resistance to both is obvious, especially where the two occur simultaneously. Apparently Rex is the only variety immediately available that meets this need. Others may be developed by combining the resistance of Golden to flag smut with the resistance of other varieties to dwarf bunt (Table 1).

The occurrence of dwarf bunt and flag smut in the same plant (Fig. 1) was observed in several varieties. Plants infected with flag smut usually do not produce heads. However, in this case many of the flag-smutted plants succeeded in sending out spikes, all of which were infested with dwarf bunt. Thus dwarf bunt infections may have restricted the deleterious effects of flag smut infection in preventing spike emergence.—State College of Washington, Pullman, Washington.

Dominance of an Actinomycete in a Soil Microflora After 2,4-D Treatment of Plants.[1] J. R. WARREN, FREDRICK GRAHAM, AND GLENN GALE.[2] During the winter of 1948–1949, thirty-two tomato plants were planted in eight 6-in. pots of unsterilized soil in the greenhouse. When the plants were approximately 1 ft. tall and well established in the soil, 16 of them were sprayed with a commercial 2,4-D preparation. When symptoms of 2,4-D injury appeared, samples were taken of soil supporting both sprayed and unsprayed plants. Dilutions of these samples were plated in beef extract-peptone agar.

The total number of colonies was lower in the plates prepared from soil supporting sprayed plants than in those prepared from soil of unsprayed plants. In the former there developed a conspicuous number of colonies of an actinomycete with rather strong antifungal properties. This organism, while undoubtedly present in soil supporting unsprayed plants, was so relatively infrequent it remained unnoticed or was not obtained at the dilutions used.

Because of the inhibiting effect of this organism on other soil fungi (Fig. 1) its antibiotic properties were tested by culturing on agar media with a number of other organisms. Among the many fungi tested and inhibited were: *Rhizoctonia solani* Kühn, *Sclerotinia cinerea* (Bon.) Wor., *Sclerotium rolfsii* Sacc., *Alternaria solani* (Ell. & Mart.) Jon. Grout, *Venturia inaequalis* (Cke.) Aderh., *Blastomyces dermatitidis* Cast., and *Epidermaphyton interdigitali* (Priestly) MacCarthy.

The actinomycete had little if any effect against bacteria as represented by *Escherichia coli* (Migula) Castellani & Chalmers, *Micrococcus pyogenes* var. *albus* (Rosenbach) Schroeter, and *Mycobacterium* sp.

[1] Carried out in part with the aid of a grant from Ayerst, McKenna, and Harrison, Ltd., 22 East 40th Street, New York, N. Y.

[2] Present address: Department of Physiology, Duke University, Durham, N. C.

Inasmuch as the taxonomic characteristics of this actinomycete do not permit its being easily assigned to any of the similar organisms described in the Sixth Edition of Bergey's Manual[3], its characteristics are given below:

On Sabouraud's agar medium young colonies are small, round, white, and glistening, of tough consistency, and somewhat embedded in the medium. Old colonies are dull gray and exude drops of a clear, colorless liquid. A zone of brown to black surrounds the colonies. The surface becomes irregular, and eventually there is a sparse growth of short aerial hyphae.

On Czapek's agar medium colonies grow slowly and are thin, white, and glistening. No pigment is produced.

On potato plugs colonies are cream colored, glistening, and slightly raised. Older colonies are glistening gray, and a black pigment discolors the plug.

FIG. 1. *Rhizoctonia solani* Kühn inhibited by soil actinomycete.

In beef-peptone broth spherical white colonies with dark centers grow in the bottom of the tube; no pellicle is formed. A brown to black pigment discolors the medium.

Nitrates and nitrites are utilized; indol is not produced; gelatin is not liquefied; paraffin is not utilized; the fungus is not diastatic, does not change litmus milk, does not ferment sucrose, dextrose, or lactose.

Mycelium is straight, nonseptate, has true branching. After about 48 hr. the mycelium begins to break into cylindrical cells. Neither endospores nor conidia have been observed.

During this study a variant was isolated which differs from the original in the following ways: No pigment on Sabouraud's agar medium, potato

[3] Breed, Robert S., E. G. D. Murray, and A. Parker Hitchens. Bergey's Manual of Determinative Bacteriology. Sixth Edition. 1948. The Williams & Wilkins Company, Baltimore, Maryland.

plugs, or in beef-peptone broth. Growth on Czapek's agar medium is excellent, spreading rapidly. Litmus milk is acidified but not coagulated.—Department of Botany, Duke University, Durham, North Carolina.

Effect of Growth-Promoting Substances on the Germination of Urediospores of Crown Rust.[1] B. PETURSON.[2] The effects on the germination of rust spores of various environmental factors, including moisture, temperature, humidity, light, oxygen, carbon dioxide, and hydrogen-ion concentration have been studied extensively, but very little information is available on the effects of growth-promoting substances. Since some of these substances, 2,4-dichlorophenoxyacetic acid for example, are now extensively used in agriculture, their effect on spore germination is of practical as well as scientific interest. Tests were therefore made to determine the effect of

TABLE 1.—*The effect of 2,4-D and 3-indole-acetic acid on germination of urediospores of crown rust*

Chemical and concentration	Percentage germination		Chemical and concentration	Percentage germination	
	Race 34	Race 4		Race 34	Race 4
3-Indole-acetic acid			2,4-D[a]		
400 p.p.m.	Tr.	Tr.	10,000 p.p.m.	Tr.	1
330	25	Tr.	7,000	Tr.	1
220	70	15	5,000	45	15
200	95	72	4,000	35	25
135	85	78	3,000	70	30
100	95	79	2,000	60	85
65	95	80	200	90	85
Check, distilled H_2O	93	80	135	90	85
			100	90	84
			Check, distilled H_2O	90	86

[a] Isopropyl ester formulation of 2,4-D.

2,4-D and 3-indole-acetic acid (heteroauxin) on the germination of urediospores of physiologic races 4 and 34 of crown rust of oats (*Puccinia coronata* Corda var. *avenae* Erikss. & Henn.).

Solutions of 2,4-D and of 3-indole-acetic acid of various strengths were prepared. Fresh spores of crown rust of oats were transferred with a dissecting needle from mature uredia on living seedling plants to drops of the solutions on microscope slides. Approximately equal quantities of spores were placed in each drop. The slides were kept in Petri dishes and moist blotting paper was placed in the bottom of the dishes to maintain high humidity. Corresponding controls were run with drops of distilled water. After 6 hr. the spores that had germinated were counted and the percentage germination recorded (Table 1).

[1] Contribution No. 1115 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. This work was carried out in the Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota.

[2] Associate Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba.

Crown rust spores germinated in rather strong solutions of both 2,4-D and 3-indole-acetic acid, particularly so in solutions of 2,4-D. A trace to 1 per cent of spores germinated in solutions of 10,000 p.p.m. of 2,4-D and the germination percentage was high in 3,000 p.p.m. The few spores that germinated in the higher concentrations of 2,4-D produced germ tubes of normal appearance. Compared to some broad-leaved plants, crown rust spores and their germ tubes are relatively insensitive to 2,4-D. Martin[3] found a concentration of 10 p.p.m. of 2,4-D toxic to citrus seedlings, and concentrations of 1 to 3 p.p.m. injurious to young parts of citrus seedlings.

The higher concentrations of both substances definitely reduced spore germination and at none of the concentrations tried did either substance stimulate spore germination or germ-tube development. Certain other fungi are apparently even less sensitive than crown rust spores to 2,4-D. Lewis and Hammer[4] tested several fungi (*Alternaria solani*, *Sclerotinia sclerotiorum*, *Fusarium lycopersicum*, and *Penicillium expansum*) to various concentrations of 2,4-D and found that saturated solutions of 2,4-D had no inhibitory effect on germination.

Since crown rust spores are much less sensitive to 2,4-D than broad-leaved plants, it may be inferred that 2,4-D, at the concentrations suitable for weed control in grain crops, would have no effect on the germination of rust spores.—Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba.

[3] Martin, J. P. The hormone weed killer, 2,4-D. California Citrograph 31: 248–265. 1946.

[4] Lewis, Ralph W., and Charles L. Hammer. The effect of 2,4-D on some microorganisms. Quar. Bul. Mich. State Col. 29: 112–114. 1946.